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БЮЛЛЕТЕНЬ ЭКСПЕРИМЕНТАЛЬНОИ БИОЛОГИИ И МЕДИЦИНЫ

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TRANSLATED FROM RUSSIAN

CONSULTANTS BUREAU, INC.

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

PUBLIC HEALTH SERVICE

BETHESDA 14, MD.

NATIONAL INSTITUTES OF HEALTH

August 15, 1958

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vol. 45 no. 3
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PHYSIOLOGY

ANALYSIS OF CUTANEOUS RECEPTION OF PRESSURE IN MAN USING THE METHOD OF CONDITIONED VASCULAR REFLEXES

COMMUNICATION IV. THE EFFECT OF VARIOUS PHARMACOLOGIC SUBSTANCES ON THE CHARACTER OF CONDITIONED VASCULAR REACTIONS DEVELOPED TO BAROSTIMULATION OF THE SKIN

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(Received June 21, 1957. Presented by Active Member AMN SSSR V. N. Chernigovskii)

It was shown in the preceding communication [10] that the cerebral cortex played a definite role in the regulation of cutaneous reception of pressure in man. The level of excitatory and inhibitory processes in the cerebral cortex and their interaction at every given moment determine both the character of the unconditioned and conditioned vascular reaction to barostimulation of the skin and the intensity of perception of the sensation caused by the stimulation. These features are very clearly brought out in the process of elaborating conditioned vascular reflexes to loads of 1 g and 500 mg, as well as in experiments with anesthesia [10].

The present work is concerned with the study of the influence of pharmacologic substances which have a selective action on the excitatory and inhibitory processes in the cortex on conditioned vascular reflexes to pressure and on the intensity of sensation produced by barostimulation.

It is known from literature that one of the most active substances with respect to inhibitory processes is bromine [4, 5, 6]. In small doses it improves conditioned reflex activity by balancing excitatory and inhibitory processes in the cerebral cortex, while in large doses it promotes spread of inhibition over the whole cortex which leads to deterioration of conditioned reflex activity [3].

We used bromine with the latter aim in view.

Phenamine (benzedrine) enhances the excitability of the cerebral cortex and improves conditioned reflex activity [2, 7]. Its effect on cutaneous baroreception was investigated.

In the first series of experiments a study was made of the effect of bromine on conditioned vascular reflexes elaborated in two subjects (T. and D.) to loads of 1 g and 500 mg, placed on the dorsal aspect of the right hand, the conditioned stimuli used being bells of different tone. The effect on conditioned vascular reflexes elaborated in subject V. to stimulation by touch of the inferior-lateral aspect of the right shoulder, the conditioned stimulus in the latter case – a load of 1 g placed on the dorsal aspect of the right hand – was also studied. The effect of bromine on the intensity of sensation produced by the barostimuli used was also determined.

The experimental method has been described in detail in Communications I and II [9, 10].

The stimuli mentioned above always produced a vasoconstrictor reaction in all the subjects investigated; this reaction began from the moment at which the conditioned stimulation was applied; a sensation of weight or touch was also constantly present during all experimental combinations. The subjects were given a 4% solution of sodium bromide 3 times a day, one tablespoonful of which corresponded to about 1.5 g pure sodium bromide per day. Bromide was administered for 6 days, i.e., each subject received a total of up to 8 g bromine.

Impairment of conditioned reflex activity was noted in the subject T. the next day after she had taken the first 1.5 g bromine; this included disappearance of the conditioned phase of the vascular reaction and considerable lengthening of the latent period of the reflex in seven out of eight combinations. The normal conditioned vasoconstrictor reaction, commencing with the sound of the bell, only appeared on the eighth combination. Sensation of weight proved to be somewhat diminished and on one occasion the subject failed to feel the load altogether.

By the 5th day, when the subject had received 6 g sodium bromide, even greater impairment of conditioned reflex activity and of sensation could be demonstrated. A load of 1 g, used without the corresponding conditioned stimulus, produced delayed and slight vasoconstriction and failed to produce a sensation of pressure (weight). On repeating the experiment (also without the bell) no vascular reaction occurred, but the sensation of weight was perceived. Of nine combinations of loads of 1 g and 500 mg with corresponding conditioned stimuli almost no vascular reaction was noted in the first 2-3 combinations (Fig. 1, a), in some combinations vasoconstriction only appeared as an after effect and only in the ninth combination, which concluded the experiment, (Fig. 1, b) did a slowly developing, slight conditioned vasoconstrictor reaction appear and increas as an after-effect. In a number of combination the perception of load was diminished.

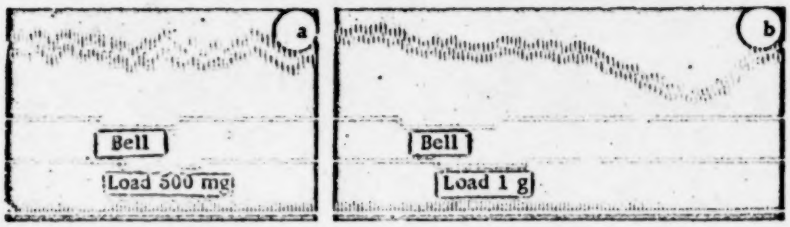

Fig. 1. Impairment of conditioned vascular reflexes in the subject T. to the combination: bell + load 500 mg (a), and bell + load 1 g (b), after administration of 6 g bromide (5th day of administration). Records from above down: plethysmogram; marker denoting conditioned stimulus; marker denoting unconditioned stimulus; time marker (2 seconds).

The same results were observed in this subject on the 6th day of bromide administration.

Similar impairment of conditioned reflexes was caused by bromide in the other subjects investigated. In the case of subject V., for whom a load of 1 g served as the conditioned stimulus and stimulation by touch as the unconditioned one, no vascular reaction occurred during the first two of six combinations (load of 1 g and touch) on the 2nd day of bromide administration, and only after the last two combinations was some slight vasoconstriction observed from the moment of application of the load. Perception of weight and touch was considerably diminished in all the combinations. During subsequent days marked inhibition of conditioned vascular reflexes was also noted; these were only restored by the last combination in the experiment; diminution of the intensity of sensation produced by the stimuli used was also observed.

On cessation of bromide administration the conditioned vascular reflexes were gradually restored, apparently running parallel with elimination of bromide from the organism. Thus, in the case of subject T., persistent impairment of conditioned vascular reflexes to load was observed on the 2nd day following cessation of bromide intake. Thrice-repeated application of the load without combination with the conditioned stimuli failed to produce a vascular reaction; perception of weight was faint. There was almost no reaction in six out of eight combinations of loads with corresponding bells. Only during the eighth combination a slight conditioned vasoconstriction reaction appeared; perception of weight was diminished. On the 3rd day the conditioned vasoconstrictor reaction became already apparent by the fourth combination. From the 4th day the conditioned vasoconstrictor reaction appeared from the first combination in all experiments. The intensity of sensation produced by the loads also underwent gradual restoration.

An approximately similar picture of restoration was noted in the case of subjects V. and D.

Restoration of conditioned vasoconstrictor reflexes towards the end of each experiment during administration of bromide suggests that the cerebral cortex can restore temporarily disrupted interrelations and coordinations even in the presence in the body of considerable amounts of a pharmacologic agent which lowers cortical excitability. Owing to the dynamic nature of cortical processes, a temporarily disrupted conditioned reflex pathway undergoes restoration on repeated application of appropriate combinations.

The second series of experiments was devoted to the study of the influence of a single dose of phenamine (0.02 g, therapeutic dose) administered 40 min prior to the beginning of experiment, on the conditioned vascular reflexes evolved to loads of 1 g and 500 mg in the subjects T. and D. and to touch in the subject V.; its influence on perception of weight was also studied.

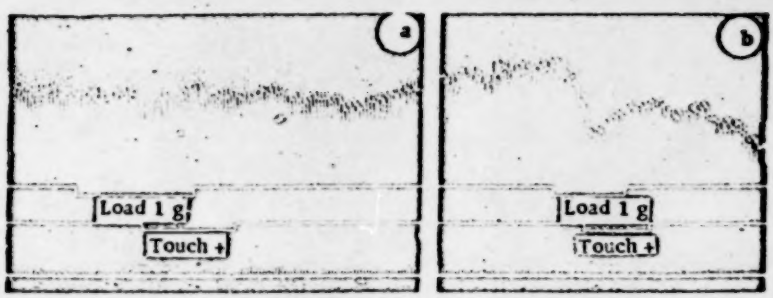

Fig. 2. Impairment of conditioned vascular reflexes in subject T. to combination "load 1 g + touch" before administration of phenamine (a) and after administration of 0.02 g phenamine (b). Records the same as in Fig. 1.

Phenamine caused a state of slight "drunkenness," vertigo, tearfulness in subject T. Forty minutes after taking the preparation, she was subjected to examination. Thrice-repeated application of 1 g and 500 mg weights without combination with conditioned stimuli produced no vascular reaction, while in the course of eight combinations with corresponding bells conditioned vasoconstrictor reactions appeared only from the fifth combination onwards. Perception of weight was present inconstantly.

Phenamine produced a similar effect on the subject V. Of nine combinations (load of 1 g and touch) vasoconstriction was associated with only the last two, beginning at the moment of application of the load (Fig. 2, b); during the seventh combination vasoconstriction started from the application of touch; in the six initial combinations there was practically no vascular reaction (Fig. 2, a).

It can thus be said that phenamine in the dose of 0.02 g exerts considerable inhibition on conditioned vascular reflexes to load and touch and also diminishes the intensity of sensation of weight. Such action of phenamine can evidently be explained by the fact that the given dose, which is generally recommended for stimulation of cerebral cortical activity, proved to be somewhat high for the subjects and produced a state of overstimulation and supra-threshold inhibition of the cortex. This would account for the absence of the expected increase in conditioned vascular reflexes and increased intensity of weight perception. But here, too, restoration of vasoconstrictor conditioned reactions was observed during the last combination in the experiment. Smaller doses of phenamine led to increased conditioned vascular reactions in the same subjects without unpleasant side-effects ("drunkenness," confusion etc.).

The same dose of phenamine had an opposite effect on the subject D. First, it produced neither vertigo nor a state of "drunkenness" in D. Secondly, the conditioned vascular reflexes to loads, which were not particularly strong in this subject prior to administration of phenamine (Fig. 3, a), were markedly enhanced after taking phenamine (Fig. 3, b). Phenamine was without effect on the intensity of weight perception by D.

The different results obtained in this investigation are being provisionally explained by individual "type features" of the subjects examined. A phenamine dose of 0.02 g proved to be too high for subjects with a more excitable nervous system (T. and V.) and gave rise to a state of supra-threshold inhibition. In the case of subject D. who showed weaker excitatory processes, phenamine only enhanced the excitatory process with consequent increase of conditioned vascular reflexes to loads..

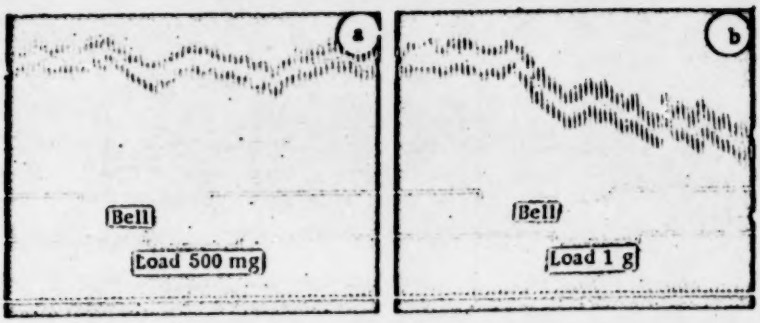

Fig. 3. Effect of phenamine (0.02 g) on magnitude of conditioned vascular reflexes to combination "bell + load of 1 g" and "bell + load of 500 mg" in subject D.
a) Before administration of phenamine; b) after administration of phenamine.
Records the same as in Fig. 1.

The importance of variations in "type features" with respect to reaction to phenamine has been pointed out by L. I. Kotliarovskii [1].

The investigation has shown that pharmacologic substances which alter the functional state of the cerebral cortex in the direction of excitation or of inhibition cause corresponding changes in conditioned reflex activity associated with barostimulation of the skin as well as the intensity of sensation produced by such stimuli.

SUMMARY

The author studied the effect of bromide and phenamine (benzedrine), which act selectively on the processes of inhibition and excitation in the cortex, on conditioned vascular reflexes to loads and to the intensity of perception of the barostimuli. Bromine caused inhibition of conditioned and unconditioned vascular reflexes to loads and decreased the intensity of perception of weights. Phenamine in the dose of 0.02 g also caused inhibition of unconditioned and conditioned vascular reflexes to weights in 2 cases and weakened the intensity of sensation. In lower doses, phenamine increased the vascular conditioned reflexes. In one case with a weaker excitability, phenamine in the dose of 0.02 g considerably increased the vascular conditioned reflexes to weights. Vascular conditioned reflexes to weight which were disturbed by bromide and phenamine returned to normal at the end of each experiment. This took place in all cases, although the pharmacological agent decreasing the excitation of the cerebral cortex was till present in the organism.

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PARTICIPATION OF THE NERVOUS SYSTEM IN THE ACTION OF SECRETIN

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(Received May 24, 1957. Presented by Academician K. M. Bykov)

Bayliss and Starling (1902) denied participation of the nervous system in the secretion-inducing action on the pancreas of both secretin and of hydrochloric acid. However, this topic remained debatable owing to the existence of contradictory data.

Much factual material has accumulated over recent years concerning the mechanism of hydrochloric acid action on the pancreas; these data indicate that hydrochloric acid solutions, on the one hand, stimulate the formation of secretin in the small intestine and, on the other hand, affect reflexly the secretory function of the pancreas. A. V. Solov'ev [6], for example, observed considerable reduction in volume of acid-induced pancreatic secretion associated with subcutaneous injection of ergotoxin. Facts pertaining to participation of the nervous system in the acid-induced pancreatic secretion have also been obtained by T. V. Serbeniuk [5], Z. V. Kobakhidze [3] and V. E. Robinson [7]. Nevertheless, up to the present some questions concerning the mechanism of secretion-inducing action of secretin on the pancreas still remain unclear. In particular, it is not clear whether secretin acts directly on the secretory tissue of the gland or affects it by a reflex mechanism.

The aim of the present work, suggested by Professor E. S. Airapet'iants was to probe the reflex component of secretin action. An attempt was made to elucidate the distinctive features of direct action of secretin on the receptors of a humorally-isolated area of the intestine connected with the organism by nervous connections only.

A brief report of the results of our first investigations was made at the Conference on Problems of Physiology and Pathology of Digestion [1].

EXPERIMENTAL METHODS

Short-term experiments were performed on cats under urethane anesthesia, using the well-known perfusion techique. The following functions were recorded during the experiments: blood pressure in the carotid artery, respiration by way of a tracheotomy tube, and volume of pancreatic secretion by means of a drop-recorder. Secretin prepared by the Bayliss and Starling method was used in most of the experiments. In control experiments secretin obtained by the method of Weaver [10] was used, as well as the crystalline secretin preparation (Eli Lilly and Company, Indianapolis).

EXPERIMENTAL RESULTS

The results of 30 experiments showed that introduction of 1-5 ml secretin into the stream of perfusion fluid gave rise to changes both in blood pressure and (in half the experiments) in respiration (Fig. 1). The area of intestine stimulated by secretin was connected with the organism by nervous connections only, so that changes in cardiovascular and respiratory activity could only be induced by reflex action of secretin. However, no pancreatic secretion could be observed even when large doses of secretin were introduced into the perfusing fluid. Nor could any appreciable differences be detected in the reflex action of secretin on using it to stimulate the

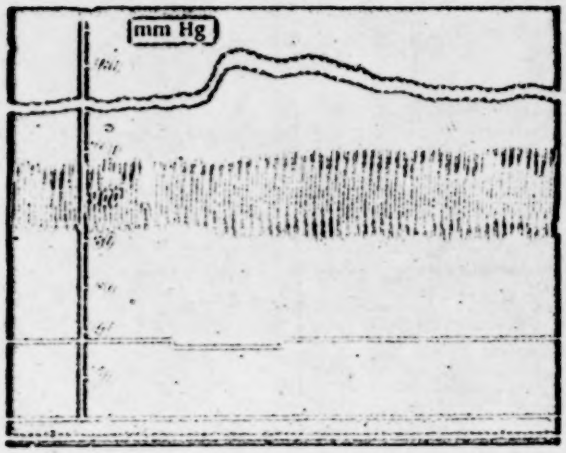

Fig. 1. Changes in blood pressure level and character of respiratory movements on introduction of 2 ml secretin (prepared by the Bayliss and Starling method) into the blood vessels of a perfused area of intestine.
Experiment No. 28, July 31, 1952. Records from above down: blood pressure, respiration, stimulus marker, base line, time marker (1 second).

receptors in different parts of the small intestine. The magnitude of changes in the blood pressure and respiration depended on the amount of secretin introduced. Thus, for example, in Experiment No. 27 (June 26, 1952) addition of 1 ml secretin to the perfusate caused a rise of 8 mm Hg in the blood pressure, and addition of 5 ml secretin increased the blood pressure by 24 mm Hg.

No changes were ever noted in the activity of the cardiovascular and respiratory systems when Tyrode solution in amounts similar to those of secretin was introduced into the perfusate; the same techniques were employed throughout. A similarly negative result was obtained when the chemical medium used for the preparation of secretin was tested (0.5% solution HCl and 10% solution NaOH at pH 6.65).

Secretin obtained by the method of Bayliss and Starling possesses vasodilator properties and special control experiments were therefore staged in which secretin free of vasodilator properties, prepared by the method of Weaver, was used. Changes in cardiovascular activity were noted in this case also. However, this considerably more highly purified secretin extract (as the mucosal extract of Bayliss and Starling) contains a series of other substances (pancreazymin, cholecystokinin) which may have, in some way, exerted a reflex influence resulting in the observed phenomena. To resolve these doubts, we resorted to Bayliss' and Starling's reference to the fact that secretin was absent in the lower part of the small intestine. It seemed to us that introduction into the perfusate of the extract of mucosa from the lower portion of the small intestine, containing all the same substances as the extract from the upper portion of the small intestine (excepting secretin) could, under the given experimental conditions, give valuable control results. The experiments, however, showed that in this case also changes occurred in respiration and blood pressure.

Subsequent experiments showed that intravenous injection of extract from the mucosa of the lower portion of the small intestine also induced pancreatic secretion. Eleven supplementary experiments were performed in order to determine the distinctive features of the secretory action of this extract; these experiments revealed that the latter's activity was one-quarter to one-fifth, on the average, of that exhibited by the extract prepared from the upper portion of the small intestine (Fig. 2). On the basis of literature data [8, 9] as well as of our experiments, it may be supposed that the mucosa of all parts of the small intestine contains

secretin, whose secretion-inducing action diminishes with distance from the duodenum. Therefore the extract from the lower portion of the small intestine could not be used in our experiments as control with respect to the observed fact of reflex influence of secretin. It was only possible to resolve doubts as to the ability of secretin to be a stimulus for receptor endings of the small intestine by performing experiments with a chemically pure, standard preparation of secretin. The results of these experiments demonstrated that a dose of 10-20 clinical units of secretin was quite sufficient to induce a rise of blood pressure when introduced into the vessels of the perfused area of the intestine (Fig. 3).

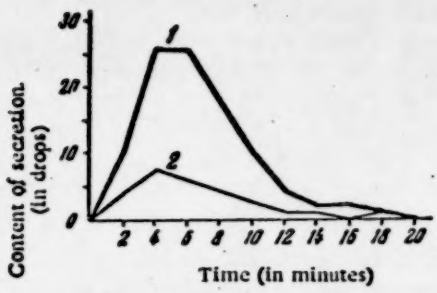

Fig. 2. Comparison of activity of secretin obtained by the Bayliss and Starling method from the mucosa of the upper portion of the small intestine (1) with the action of secretin obtained by the same method but from the lower portion of the small intestine (2). In both cases 5 ml secretin was injected intravenously. Experiment No. 35, August 8, 1952.

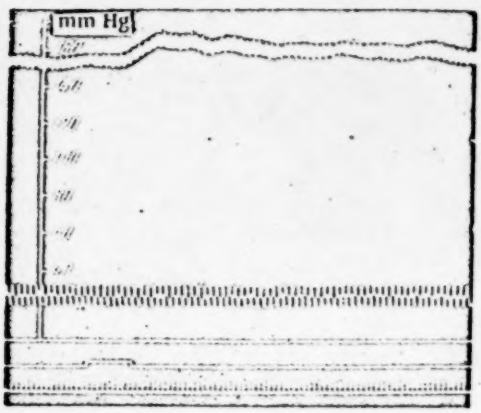

Fig. 3. Changes in blood pressure level and respiration in experiment with introduction of 20 clinical units of a chemically pure standard preparation of secretin into the vessels of a perfused area of the intestine. Records from above down: blood pressure, respiration, base line, marker denoting stimulation, time marker (1 second). Experiment No. 67, February 8, 1957.

In 1946 V. A. Alekseev [2], using the pancreatic perfusion technique, observed changed in blood pressure and respiration on introduction of secretin into the pancreatic vessels, i.e., he demonstrated the action of secretin on the receptor apparatus of the pancreas. Analogous work on the histological plane was carried out by L. V. Oleandrov [4]. The author concluded from the evidence of simultaneous staining of secretory and nervous tissue of the pancreas under the influence of intravenously administered secretin that the latter acted not only on the secretory cells of the pancreas but also on its local nervous centers. The data obtained in our experiments should be considered in the light of data reported by the above-mentioned authors. The results of the present investigation indicate that secretin, being an intestinal chemoreceptor stimulant, excites the sensory endings embedded in the walls of the small intestine at the site of its formation.

Material demonstrating the regulatory significance of these reflex influences on the functional state of the pancreas is presented in another communication.

SUMMARY

The effect of secretin on the interoceptors of the small intestine was studied in short-term experiments.

It was demonstrated that introduction of secretin into the vessels of the perfused area of intestine connected with the organism by the nerves alone caused changes in the activity of the cardiovascular and respiratory system by reflex pathways. The results of these experiments give grounds for concluding that secretin stimulates the intestinal chemoreceptors.

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COMPARATIVE DATA ON CHANGES IN HIGHER NERVOUS ACTIVITY IN DOGS WITH FALSE AND TRUE PREGNANCY

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(Received February 6, 1957. Presented by Active Member AMN SSSR P. S. Kupalov)

Much work has been devoted to the question of false pregnancy. Changes in the sexual apparatus, mammary glands and even behavior in animals with false pregnancy resemble in many respects those associated with true pregnancy [1, 3-9]. As far as we know, no special investigation of higher nervous activity in false pregnancy had been carried out.

Comparison of unconditioned and conditioned reflexes during false and true pregnancy is essential for clarifying the mechanism of changes in nervous activity characteristic for this period. Changes which are caused by the action of ovarian hormones should be present in both cases; however, those changes which are caused by stimulation of sexual organ receptors by the developing fetus and placental influences should be absent in cases of false pregnancy.

Dogs form a suitable object for such observations. The duration of false pregnancy in dogs is similar to that of true pregnancy, viz. 63-65 days. The corpus luteum develops and functions during 30-35 days and then undergoes involution.

EXPERIMENTAL METHODS

Experiments were carried out in the course of 3 years, using classical Pavlovian technique; 4 dogs with different types of higher nervous activity were used. All the experimental animals were young and had not had any pregnancies. "Pushka" exemplified the well-balanced type of nervous activity. Of the other dogs one was as nervously strong as Pushka, the remaining two were weaker.

Two and 3 true pregnancies were traced in each dog, and in the case of Pushka a false pregnancy was studied first, then 3 true pregnancies.

The following stereotyped pattern of conditioned stimuli was used in the experiments: bubbling noise, light, metronome with a rate of 120 strokes per minute (M_{120}), metronome with rate of 60 strokes per minute (M_{60} – differentiation), metronome with rate of 120 strokes per minute (M_{120}) and bell. Duration of isolated action of conditioned stimuli was 20 seconds, followed by reinforcement with meat-biscuit powder, with the exception of M_{60}. The dogs' behavior and their reaction to the conditioned stimuli and to food were recorded (motor-food reactions I and II).

EXPERIMENTAL RESULTS

On March 5, 1953, Pushka was covered, but no pregnancy ensued. At the same time, on the 44th day

after this (as in true pregnancy) swelling of the mammary glands was noted, and on the 60-61 day appearance of colostrum was observed. Puska's behavior underwent a change with the onset of false pregnancy which was similar to change observed both in her and in other dogs during true pregnancy. During two and half weeks Pushka was restless in the course of experiments and made attempts to lie down. Isolated conditioned stimuli induced yawning. She did not refuse food.

During the next three weeks the dog was sluggish, lying down most of the time, barely lifted its head in response to conditioned stimuli, rose slowly when given a bowl with meat-biscuit powder and began eating. After the meal she again lay down. At this time she sometimes refused food. Such manifestations were more marked during true pregnancy, particularly refusal of food.

Beginning from the 6th week of false pregnancy Pushka became lively, reacted quickly to conditioned stimuli, took her food at once and only on the day of supposed confinement (64th-65th day) was a little sluggish.

At the onset of false pregnancy the unconditioned salivary secretion began to diminish gradually (Fig. 1), reaching a minimum on the 25th day of false pregnancy (115 divisions on the scale). Prior to false pregnancy the unconditioned salivation amounted to an average of 202 divisions on the scale.

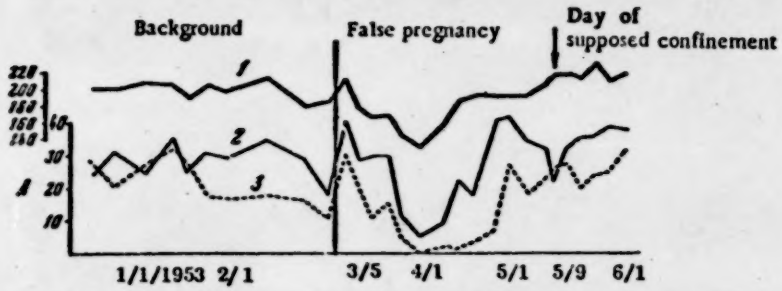

Fig. 1. Changes in magnitude of unconditioned and conditioned reflexes in false pregnancy. Records: 1) magnitude of unconditioned reflexes; 2) magnitude of conditioned reflex to bubbling noise; 3) magnitude of conditioned reflex to a bell.

After the 25th day of false pregnancy the unconditioned salivation began to increase gradually and by the 33rd day had already reached the initial level.

During true pregnancy the magnitude of unconditioned reflexes either remained at the previous level or tended to increase to some extent (Fig. 2).

The increase of unconditioned reflex salivation during pregnancy was even more pronounced in the other dogs. The course of unconditioned salivation prior to pregnancy was always regular, i.e., the maximal secretion of saliva occurred during the first 30 seconds from the beginning of the meal; there was a decrease in salivation by the end of the 1st minute; with the onset of true pregnancy, however, although the magnitude of unconditioned reflexes remained practically at the previous level, the course of unconditioned salivation showed a distinct change. There was a decrease in secretion during the first 30 seconds of feeding and an increase during the following 30 seconds (Fig. 3). These changes were maintained not only up to the time of confinement but were also observed at the beginning of the period following parturition.

A different picture was observed in false pregnancy. Despite the decrease in the magnitude of unconditioned reflexes the course of unconditioned salivation was altered only very slightly and transiently. Such changes were only observed on the 20th-34th day of false pregnancy (Fig. 3).

Changes in conditioned reflexes during false and true pregnancy had both common features and certain differences. Similarities consisted of diminution of conditioned reflexes towards the first half of both true and false pregnancy. For example, the conditioned reflex to a bubbling noise decreased during false pregnancy

from 29 divisions of the scale to 8, reflex to light from 17 divisions to 2. During true pregnancy the conditioned reflex to a bubbling noise diminished from 35 divisions of the scale to 8 and the reflex to light from 26 to 2. Decreases in other conditioned reflexes were likewise quite definite.

The difference in the dynamics of conditioned reflexes consisted of the fact that in false pregnancy restoration was fairly rapid and beginning with the 40th-45th day the level of all conditioned reflexes did not differ from the initial.

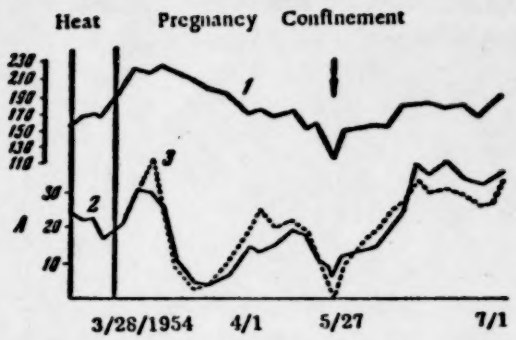

Fig. 2. Change in the magnitude of unconditioned and conditioned reflexes in true pregnancy. Records the same as in Fig. 1.

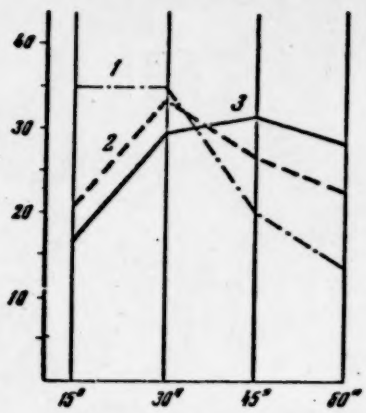

Fig. 3. Change in unconditioned secretion every 15 sec of the 1st minute in a nonpregnant animal (1), during false pregnancy (2) and during true pregnancy (3).

In true pregnancy, however, conditioned reflexes increased very slightly and did not reach the initial level during the second half. Restoration of conditioned reflexes only occurred after parturition.

Investigation of the changes in higher nervous activity during false and true pregnancy brings out 3 points requiring discussion: first, preservation of the previous level of unconditioned salivation (or even its increase) during true pregnancy and its marked decrease during false pregnancy; secondly, comparable decrease of the magnitude of conditioned reflexes in the first half of false and true pregnancy and thirdly, rapid restoration of conditioned reflexes towards the end of false pregnancy and maintenance of a low level of such reflexes in true pregnancy.

According to data available in the literature [2], the increase of unconditioned secretion during pregnancy is explained by the influence of the corpus luteum hormone on the central nervous system. Our data indicate that this phenomenon cannot be explained by the influence of the corpus luteum hormone alone; the corpus luteum develops and functions both during true and false pregnancy, yet in the latter case the unconditioned reflexes not only did not increase but showed a clear diminution. Evidently, the corpus luteum is not the only factor responsible for the increase of unconditioned secretion in true pregnancy. Other factors, present in true but absent in false pregnancy, must apparently be involved. The stream of afferent impulses from the receptors of the sexual apparatus resulting from the developing fetus must be regarded as one of such factors.

Diminution of conditioned reflexes during the first half of false and true pregnancy suggests some common cause responsible for the phenomenon. Such marked decrease in the magnitude of all conditioned reflexes characterizes a state of inhibition developing in the cerebral cortex. The course of unconditioned salivation which approaches the subcortical type at this time indicates that the inhibition spreads not only to the cortical part of the analyzers but also to the cortical representation of the unconditioned food reflex. In true pregnancy the change in the course of unconditioned salivation proved to be more profound, which suggests that inhibition of the cerebral cortex was more extensive.

The causes underlying cerebral cortex inhibition may possibly depend on the state of sexual excitation and hormonal influences preceding pregnancy; moreover, in true pregnancy the developing fetus must play an important role.

During the second half of pregnancy when the secretion of the corpus luteum hormone diminishes and the fetus grows considerably with consequent increase in afferent stream of impulses from the uterine receptors, distinct differences in the dynamics of conditioned reflexes begin to be apparent in false and true pregnancy.

Furthermore, in true pregnancy, the hormonal influences of the corpus luteum are supplemented by the action of placental hormones. Apparently as the result of these influences the conditioned reflexes remain lowered during the second half of true pregnancy; they return to the initial level only after parturition.

In false pregnancy the whole complex of stimuli associated with the developing fetus and the action of placental hormones is absent and the conditioned reflexes return rapidly to the original level.

SUMMARY

Development of inhibition in the cerebral cortex was noted during the first half of both true and false pregnancies. This is explained by production of lutein by the ovaries. In true pregnancy this inhibition may be enhanced by the afferent impulses coming from the receptors of the uterus when these receptors are stimulated by the developing fetus. It was demonstrated that placental hormones may affect the condition of the higher nervous activity during the second half of pregnancy.

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SUBORDINATING EFFECT OF THE DIENCEPHALON ON ACCOMMODATION OF THE SCIATIC NERVE

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D. N. Nasonov and collaborators have recently [3] questioned the existence of subordinating influences of the central nervous system on the physiologic properties of the peripheral nerve trunks. Their views were based on the fact that subordinating influences had been demonstrated by the use of transection or ligation of the nerve. Since such interference with the nerve represents not only interruption of connection of its distal part with the central nervous system but also a traumatic influence inflicted on the nerve itself, the changes in the physiologic properties of the nerve induced by such procedure may be the result not only of interruption of its connection with the brain but also of trauma to the nerve itself.

The question concerning the existence of subordinating influences of the central nervous system on the nerve can only be resolved by such experiments in which action is directed against the central nervous system with preservation of its connection with the nerve under investigation, i.e., without transection or ligation of the latter.

Experiments of this sort have been described by P. E. Motsnyi [2] who concludes that Sechenov inhibition as well as direct stimulation of the sympathetic trunk in frogs lead to lowering of accommodation of the sciatic nerve. However, the conclusions made by the author do not follow from his data as we found on analysis of the myograms presented in his paper. Motsnyi assesses the accommodation of the nerve by the ratio of two values – the height of muscular contraction in a nerve-muscle preparation in response to stimulation of the nerve with square-wave current (N_s) and the height of such contraction in response to stimulation of the nerve by exponential current of a certain height (N_e), i.e., $N_s : N_e$.

As the author mentions, N_s is an index of excitability and N_e an index of accommodation. This method is permissible, although it is more expedient to measure accommodation not with a single gradient of current rise, but several, since that determins the whole accommodation curve. But when such a method is used it is necessary to take into account that a change in the ratio $N_s : N_e$ indicates a change is brought about by a change in N_e. The myograms presented in Motsnyi's work however, show that in his experiments N_s and not N_e underwent changes, while the latter remained almost unchanged. Consequently, the changes described in Motsnyi's paper refer not to accommodation but to the excitability of the nerve on square-wave stimulation. This, of course, does not exclude the possibility that changes in accommodation also took place; but determination of several points along the accommodation curve, which was not done by the author, is necessary for detailed judgement.

The question regarding subordinating influence of the higher centers on the rate of accommodation of peripheral nerve thus remains open and still requires experimental confirmation.

EXPERIMENTAL METHODS

In our experiments the subordinating influence of the higher centers on the accommodation of peripheral nerve was investigated with determination of the accommodation curve using 4 gradients of stimulating current

rise, viz.: successive connection of capacities of 1/1000, 1/100, 1/10 and 1μ f. Prior to determination of the excitability threshold of the nerve with respect to these gradients its threshold with respect to square-wave stimulation (rheobase) was measured; minimal contraction of the muscle served as index. Experiments were performed on thalamic frogs (extirpation of cerebral hemispheres by the I. M. Sechenov method). We studied changes in accommodation in the presence of Sechenov inhibition (application of a crystal of rock-salt to the sectioned optic thalami for 2 minutes) and also on stimulation of the skin of the dorsal surface of the shin by applying to it a piece of filter paper (1 × 1 cm) moistened with a 0.5% solution of sulfuric acid. The preparation was placed into a moist chamber; the experiments were begun 1-1½ hours later. In the early experiments stimuli were applied at 1 minute intervals during the determination of the rheobase and accommodation; in subsequent experiments half-minute intervals were used since this did not affect the values obtained.

EXPERIMENTAL RESULTS

Our experiments demonstrated that in the presence of Sechenov inhibition marked lowering of accomodation takes place, chiefly with respect to stimulating currents of small gradient; with capacities of 1/10 and 1μ f the threshold is halved and more. The rheobase is lowered slightly, by approximately 25-30%. Unlike P. E. Motsnyi, we observed these changes over a prolonged period; after removal of the salt crystal from the sectioned optic thalami the changes continue to grow and then slowly diminish and return almost to the initial values after 40-60 minutes (Fig. 1, A). The fact that Motsnyi observed a change in the ratio $N_s : N_c$ only during stimulation of the sectioned optic thalami is probably accounted for by the fact that he did not determine the whole accommodation curve but only one of its points which, moreover, was close to the rheobase.

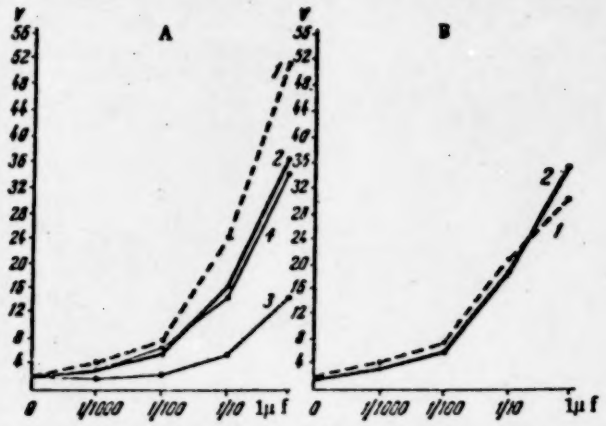

Fig. 1. Effect of Sechenov inhibition on accommodation of the sciatic nerve in frog.
A) Thalamic preparation. Nerve accommodation curve; 1) before and 2) during Sechenov inhibition; 3) 10 minutes after and 4) 25 minutes after removal of rock-salt from optic thalami. B) Thalamic preparation. Similar experiment with nicotine-blocked sympathetic trunk; 1) before and 2) during Sechenov inhibition.

Slowing of accommodation in Sechenov inhibition is also noted when the spinal cord is transected in the lower thoracic region. Following painting of the sympathetic trunks with a 1% solution of nicotine, Sechenov inhibition no longer induces a change in nerve accommodation (Fig. 1, B); in this respect our data diverge from Motsnyi's data who continued to observe the described phenomenon after section of all r.r. communicantes leading to the sciatic nerve.

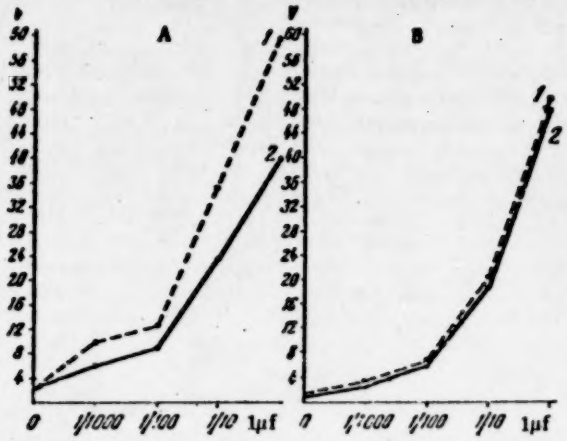

Fig. 2. Effect of cutaneous stimulation (shin) with 0.5% solution of sulfuric acid on accommodation of the sciatic nerve in frog. A) Thalamic preparation. Accommodation curve for the nerve: 1) before and 2) during cutaneous stimulation. B) Bulbar preparation. Nerve accommodation curve: 1) before and 2) during cutaneous stimulation.

In experiments in which the skin of the frog's leg was stimulated with acid, as in Sechenov inhibition, lowering of accommodation was observed (Fig. 2, A). This effect is not observed when the experiment is performed on a bulbar or spinal preparation rather than on a thalamic one (Fig. 2, B).

A study was also made of changes in accommodation of the sciatic nerve which occur on nicotine-blocking of the sympathetic trunk in the absence of stimulation of optic thalami and skin of the leg. It was found that exclusion of the sympathetic trunk in itself lowered accommodation; the effect however was slight. It was observed only on the side ipsilateral to the affected sympathetic trunk; accommodation of the contralateral nerve remained unchanged (Fig. 3, A). The effect of destruction of the spinal cord with preservation of the brain stem was also investigated; the sympathetic trunk was left intact in these experiments. Such interference with the central nervous system did not alter the accommodation of the sciatic nerve (Fig. 3, B).

The present series of experiments, using accommodation of the sciatic nerve as an example, has proved that the functional state of peripheral nerve trunks is, contrary to the opinion of D. N. Nasonov and his collaborators, subordinated to the central nervous system. The subordinating influences on accommodation of the sciatic nerve demonstrated by us originate in the diencephalon (which, naturally, does not exclude the possibility of the existence of such influences in other, more particularly higher, parts of the central nervous system).

The fact that lowering of sciatic nerve accommodation upon stimulation of the optic thalami does not occur if the sympathetic trunk is excluded by nicotine suggest that the subordinating influence of the diencephalon on accommodation of this nerve is mediated by the sympathetic pathway (fine nonmedullated nervi nervorum of the type of Timofeev's fibers described by I. V. Torskaia [7]).

The diencephalon exerts a subordinating influence on sciatic nerve accommodation not only in the presence of stimulation of the optic thalami and leg skin, but also in the absence of these stimuli. This is demonstrated by those experiments in which the sympathetic trunk was excluded from the sphere of action in frogs which had not been subjected to such stimulation. Judging by the fact that such exclusion of the sympathetic trunk, as well as stimulation of the optic thalami and leg skin, lead to lowering of sciatic nerve accommodation (although less marked in the former case), it may be concluded that the diencephalon subordinates sciatic nerve

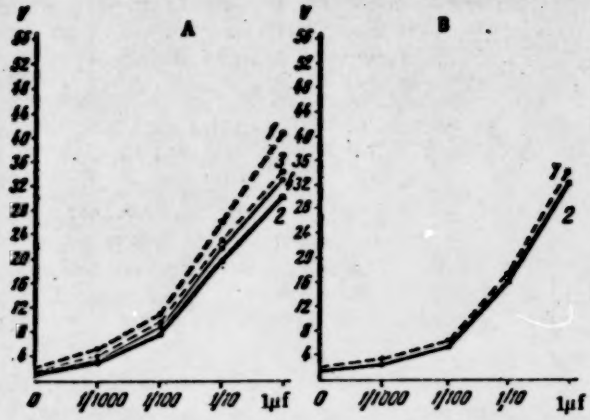

Fig. 3. Effect of exclusion of the sympathetic trunk and destruction of the spinal cord on sciatic nerve accommodation in frog.
A) Thalamic preparation; 1) and 2) accommodation curves for the right sciatic nerve before and after exclusion of the right sympathetic trunk by means of nicotine; 3 and 4) corresponding accommodation curves for the left sciatic nerve. B) Thalamic preparation. Sciatic nerve accommodation curve: 1) before and 2) after 10 minutes following destruction of the spinal cord. Sympathetic trunk preserved.

accommodation when the diencephalon is not stimulated; exclusion of the sympathetic trunk abolishes this subordinating influence.

The character of the subordinating influence exerted by the diencephalon on sciatic nerve accommodation thus depends on the functional state of the diencephalon; in a state of rest the diencephalon maintains a high degree of sciatic nerve accommodation but lowers such accommodation when it is in a state of activation.

The possibility is not excluded that lowering of accommodation is an adaptive-trophic effect which ensures adaptation of the nerve to the working requirements of the organism; lowering of accommodation, as shown by L. V. Latmanizova [1] is associated with increase in lability ensuring adoption of the rhythm of inflowing impulses by the nerve. This point of view is supported by the subordinating influences of the diencephalon on the sciatic nerve being mediated by the sympathetic pathway. It is known that the same pathway has been found by A. V. Tonkikh to be involved in the Sechenov inhibition of the spinal cord.

The absence of change in sciatic nerve accommodation on destruction of the spinal cord with conservation of the diencephalon also indicates that the subordinating effect of the central nervous system on accommodation of peripheral nerve trunks is sympathetic in character.

The data mentioned above suggest that the subordinating influence of the central nervous system on accommodation of peripheral nerves is one of the manifestations of the universal adapative-trophic action of the sympathetic nervous system, as established by the school of L. A. Orbeli.

The present experiments permit the conclusion that Sechenov inhibition lowers the rate of sciatic nerve accommodation sharply and for long periods (20-40 minutes). This effect is also observed in preparations subjected to preliminary transection of the spinal cord in the thoracic region, but is absent when the sympathetic trunks are blocked by nicotine.

Cutaneous stimulation achieved by application of a sulfuric acid solution to the shin of a thalamic frog results in the reflex lowering of sciatic nerve accomodation. This effect is absent in bulbar and spinal preparations.

Nicotine treatment of the sympathetic trunk by itself also causes some lowering of sciatic nerve accomodation, but the effect is slight. It is only observed ipsilaterally.

Destruction of the spinal cord does not affect the sciatic nerve accommodation in a thalamic preparation.

The facts cited prove that accommodation of peripheral nerve trunks is subordinate to the central nervous system. This subordination is achieved by the diencephalon and is mediated by the sympathetic trunks; it is evidently of adaptive-trophic significance.

SUMMARY

It was established on thalamic frogs operated by I. M. Sechenov's method that accommodation of peripheral nerves is subordinate to the central nervous system and depends on the functional condition of the diencephalon. The latter enhances it in conditions of rest and decreases it during activity (Sechenov's inhibition, reflex excitation). This subordination is carried out by the diencephalon by way of the sympathetic trunks and evidently has an adaptive trophic significance.

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THE MECHANISM OF INSULIN ACTION

COMMUNICATION V. EFFECT OF INSULIN ON BLOOD GLUCOSE CONTENT IN DOGS DURING ELECTROSLEEP

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One of the present authors presented experimental data concerning the role of the cerebral cortex in the mechanism of insulin action in communications published previously [3-7]. The influence of insulin on the blood sugar level and development of the hypoglycemic syndrome was studied during inhibition of the higher regulatory centers by narcotics as well as upon surgical removal of the cerebral cortex and direct application of the hormone to nerve centers. In connection with this work it was thought interesting to study the development of the insulin syndrome during inhibition of the cerebral cortex induced by weak, rhythmic electric impulses, i.e., during electrosleep. This is particularly important because electrosleep approaches natural sleep in its physiologic characteristics [2].

There are references in the literature indicating that electrosleep does not exert any appreciable effect on carbohydrate metabolism and that the blood sugar content does not leave the limits of physiologic fluctuations [1, 2].

The present work is concerned with the study of the glycemic curve under conditions of electrosleep on the one hand, and elucidation of the effect of electrosleep on the development of the insulin syndrome during hyperinsulinism on the other hand.

EXPERIMENTAL METHODS

Dogs were used in the experiments following 16-18 hours of fasting. A preliminary determination of the blood sugar curve was made under normal conditions and after administration of insulin, samples being taken over a period of 3-6 hours. The blood glucose content was then studied during electrosleep and after administration of insulin (from 1-2 to 5-10 units per 1 kg body weight) against the background of electrosleep.

Electrosleep was induced by means of an apparatus constructed in the laboratory by Iu. N. Kasatkin. As can be seen from the diagram (see Fig.; stage I) the generator of square-wave pulses mounted on tube 6N8 (L_1) is on the lines of an asymmetric multivibrator with positive shift which increases stability of the multivibrator performance. Regulation of the frequency of the multivibrator is achieved by changes in the magnitude of the resistance R_3. Impulses from the multivibrator (from the left half of the 6N8 tube) pass through the condenser C_3 to the grid of tube $6L_6$ (L_2). Impulse amplifier (stage II) is mounted on tube $6L_6$ (L_2) whose anode circuit includes resistance R_7 which is the anode load. The whole circuit is supplied by a rectifier (stage III) assembled on the two-half period system on tube 5Ts4S (L_5) whose output is balanced by two tubes 5G-4S (L_3 and L_4). Balanced voltage is equal to 300 v. This construction permits smooth regulation of frequency within the range of 50-200 cps with constant duration of 0.3 milliseconds and a duty ratio of 1 : 10. The object is put in parallel with the anode load in series with the milliammeter graduated to 10 ma. The apparatus described, based on the multivibrator, is simple to construct and reliable in performance.

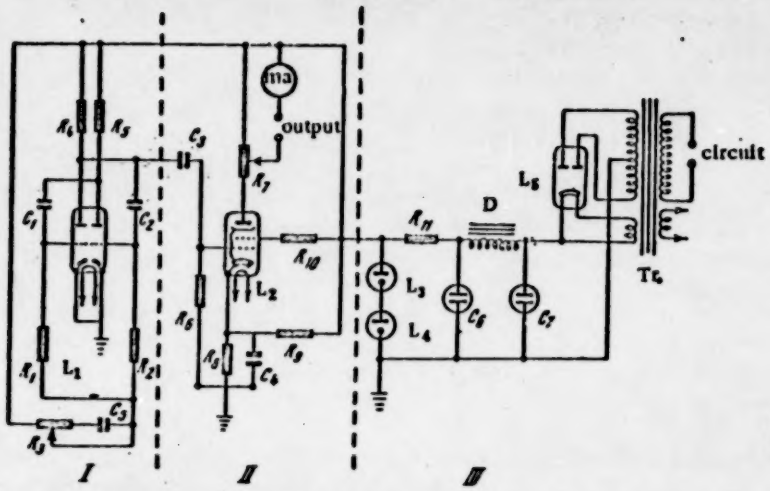

Diagram of apparatus used for inducing electrosleep. L_1-L_5) tubes; R_1-R_{11}) resistances; C_1-C_7) condensers; ma) milliammeter; Tr) transformer; D) inductor; I-III) stages.

Beofre switching on the apparatus, the electrodes (2.5 × 4.5 cm) were attached in the following way: the positive electrode was placed on the occiput, the negative on the frontal bone, its lower edge between the orbits. After establishing a certain frequency (100-150 impulses/sec) the current strength was gradually raised to 7-12 ma. In some sessions the current strength was raised rapidly to 20 ma with subsequent reduction to 5-10 ma. Later, experimental conditions were selected individually for each dog. In most experiments, sleep of sufficient depth ensued after 8-10 minutes following switching on of the current.

Blood for analysis was taken from the ear. Blood glucose content was determined by the Hagedron-Jensen method.

EXPERIMENTAL RESULTS

Experimental results proved to be consistent and therefore only part of the material is presented in Tables 1 and 2. Table 1 shows that the blood glucose level during 3 hours of electrosleep does not pass the limits of physiologic fluctuations.

TABLE 1

Fluctuations in Blood Glucose Level Under Normal Conditions and During Electrosleep

No. of experiment	Date of experiment	Experimental conditions	Blood glucose content in mg %					
			initial level	from start of experiment (in min.)				
				15	30	60	120	180
1	4/12 1957	Normal	87	86	83	90	92	89
	4/13 1957	Electrosleep	93	98	96	101	91	98
	4/18 1957	»	82	86	86	89	97	91
3	4/24 1957	Normal	93	87	94	89	87	91
	4/25 1957	Electrosleep	102	94	92	97	100	105

The influence of insulin on blood glucose level during electrosleep is illustrated by data in Table 2 which show that marked hypoglycemia developed when insulin was administered to animals during electrosleep.

It must be noted that convulsive seizures occurred in normal animals under ordinary conditions within 2-3 hours from the time of administration of large doses of insulin, whereas during electrosleep administration of the same dose of insulin was not accompanied by convulsions although the blood glucose level dropped to 30-40 mg%. However, 1-2 hours after cessation of electrosleep (5-6 hours after administration of large doses of insulin) there was a gradual onset of the main manifestations of hyperinsulinism – salivation, dyspnea, immobility. In isolated cases these manifestations were joined by convulsions. We did not succeed in observing enhancement and prolongation of the hypoglycemic action of insulin against the background of electrosleep as indicated by N. I. Akishina.

TABLE 2

Influence of Insulin on Blood Glucose Level During Electrosleep

Date of experiment	Date of experiment	Dog's weight in kg	Experimental conditions	No. of units of insulin admin.	Blood glucose content in mg%					
					before insulin administration	after insulin administration (in minutes)				
						15	30	60	120	180
1	4/21/57	8.1	Control (without sleep)	8	101	91	56	53	51	51
	4/23	8.0	Electrosleep	8	99	87	65	62	57	51
	5/9	8.3	Control (without sleep)	40	102	89	55	50	45	39
	5/10/57	8.2	Electrosleep	40	98	91	57	60	47	47
3	5/8	11.8	Control (without sleep)	110	103	99	100	63	47	36
	5/7	11.8	Electrosleep	110	107	102	99	76	59	45
	5/4	10.5	Control (without sleep)	55	88	71	43	37	30	—
6	5/6/57	10.4	Electrosleep	55	92	84	54	39	34	32

The results of these investigations demonstrate that electrosleep does not affect the hypoglycemic action of insulin. During electrosleep the insulin-induced hypoglycemia is not accompanied by the characteristic manifestations of hyperinsulinism.

SUMMARY

Variations in the concentration of blood glucose are not above or below the level of the physiological changes during electrosleep induced by weak rhythmical electric impulses. Electrosleep has no effect on the hypoglycemic action of insulin. However, when massive doses of insulin are introduced the characteristic symptoms of hyperinsulinism do not appear. A new design for a more stable apparatus for inducing electrosleep is presented.

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THE ROLE OF HYALURONIDASE IN THE PROCESS OF URINARY EXCRETION

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The problem of mucolytic enzymes, particularly the enzyme system of hyaluronidase-hyaluronic acid, has attracted the attention of research workers from various points of view. Published experimental and clinical data provide evidence for the role of this system in membrane permeability, in the physiology and pathology of connective tissue, etc.

The least known role of hyaluronidase is its participation in the regulation of water-salt metabolism. Only isolated observations are available in this respect which indicate that the mucolytic system is involved in the active binding of water and electrolytes [2-6].

As already reported [1] the enzyme hyaluronidase has been found in the urine of man and animals; this work was carried out in the laboratory of A. G. Ginetsinskii. It was established that the hyaluronidase activity of the urine was strictly dependent on the extent of diuresis, showing a decline along a characteristic curve following hydration.

The present investigation is concerned with further studies of this question.

EXPERIMENTAL METHODS

Experiments were performed on dogs with ureters exteriorized in abdominal skin flaps. The hyaluronidase activity of the urine was determined viscosimetrically, the method being based on the ability of hyaluronidase to depolymerize hyaluronic acid with consequent lowering of its viscosity. The enzyme activity was expressed in conventional units: 1 conventional unit corresponding to the activity of a preparation causing a 1% drop in the viscosity of the initial solution of hyaluronic acid in the course of 20 minutes.

EXPERIMENTAL RESULTS

In attempting to analyze the cause of decline in hyaluronidase activity of the urine with increase in diuresis, the idea first presents itself that the activity drops as the result of dilution of the enzyme entering the lumen of the nephron in some constant concentration. However, appropriate investigation showed that lowering of enzyme activity observed during increasing urinary excretion could not be explained by a concentration effect. The hyaluronidase activity of urine obtained with small diuresis remained relatively high on dilution of urine in vitro and dropped to zero only when a considerably greater dilution than that observed in natural increase of diuresis was employed (Fig. 1).

Nor is the value of hyaluronidase activity appreciably altered by concentration of urine collected during massive diuresis. If the initial sample is inactive, no activity can be detected on concentrating the urine to a volume corresponding to a low level of urinary excretion.

Experiments in which urine was dialyzed and reduced under vacuum to a volume corresponding to a diuresis of 0.2 ml/min × m^2 also indicated that the curve denoting the dependence of hyaluronidase activity did not result from changes in concentration. The use of this method of analysis excluded the influence of dilution and of a possible salt effect in every sample.

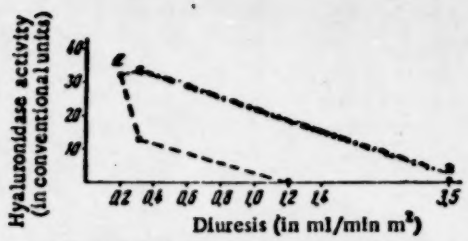

Fig. 1. Effect of dilution on the hyaluronidase activity of urine.
○) Hyaluronidase activity of urine under natural conditions; □) hyaluronidase activity of sample a after dilution of the urine to volumes corresponding to diuresis of 0.3; 1.2 and 3.5 ml/min × m².

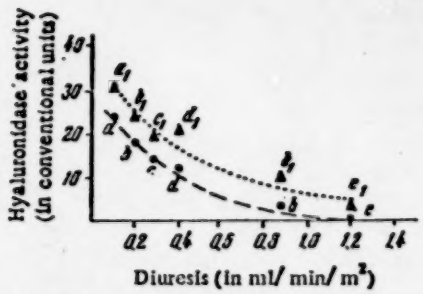

Fig. 2. Dependence of hyaluronidase activity on the magnitude of diuresis after dialysis and concentration of urine to a constant volume.
○) Hyaluronidase activity of urine under natural conditions; △) hyaluronidase activity of urine under standardized conditions. Sample a_1 obtained by dialysis of urine collected during diuresis of 0.12 ml/min × m² and addition to it of distilled water to correspond to the volume 0.2 ml/min × m². Sample b_1 obtained by dialysis of urine collected during diuresis of 0.2 ml/ min × m². Samples c, d, e_1 obtained by dialysis of corresponding samples of urine collected during diuresis exceeding 0.2 ml/ min × m² and reduced to this volume by concentration under vacuum.

Figure 2 demonstrates one of the typical experiments of this series. It shows that hyaluronidase activity of urine under standardized conditions changes with the same consistency as under natural conditions but the curve is situated at a higher level owing to removal of concentration and salt effects. This indicates that true diminution of enzyme liberation by renal tissue occurs as increased urinary excretion develops. In order to discover the nature of the processes underlying changes in hyaluronidase activity of urine we were interested in finding out whether the relationship established by us persisted during development of diuresis induced by other factors than hydration. An osmotic diuretic agent – urea – was used for this purpose.

The osmotic load was given to the dogs per os on the basis of 0.5 g urea per 1 kg body weight. The hyaluronidase activity was determined after dialysis and adjustment of the urine to a volume corresponding to a diuresis of 0.2 ml/min × m².

It is known that water diuresis develops as the results of diminished facultative reabsorption of water. Increased diuresis following urea administration however, has a different mechanism. Urea which enters the filtrate in large quantities binds the water osmotically, causing its retention in the tubules. In this case the extent of diuresis is determined by the degree of diminution of obligatory reabsorption.

Urinary excretion under the influence of osmotic diuretics increases not as the result of reduced facultative reabsorption as in water diuresis but as a consequence of inflow into the distal segment of the nephron of a volume of fluid in excess of that which can be reabsorbed despite the maximal intensity of processes occurring there.

The fall in hyaluronidase activity of the urine is thus connected not with the fact of increased urinary excretion itself but with the physiologic mechanism responsible for the development of increased diuresis. Low hyaluronidase activity – and at a certain critical level, zero activity – is associated with water diuresis which occurs under conditions of suppressed facultative reabsorption. Osmotic diuresis, on the other hand, occurs under conditions of intensive facultative reabsorption and is associated with high hyaluronidase activity of urine (Fig. 3).

These data permit the conclusion that the system hyaluronidase–hyaluronic acid participates in the process of water reabsorption which takes place in the distal segment of the nephron.

The main factor responsible for stimulation of water transport in the distal segment is known to be the antidiuretic hormone of the hypophysis. It therefore appeared wise to investigate the influence of this hormone on the hyaluronidase activity of urine.

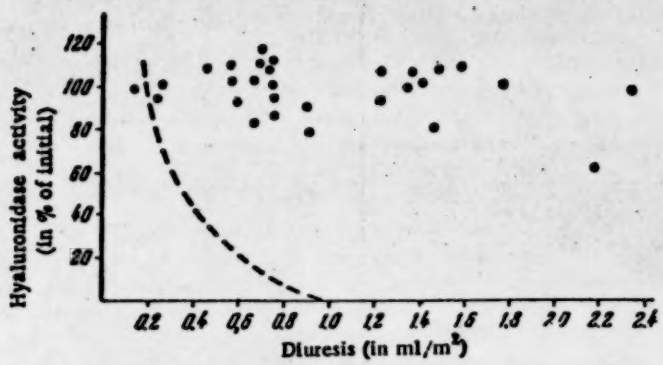

Fig. 3. Relation of hyaluronidase activity of urine to magnitude of urinary excretion during osmotic diuresis.
Broken line denotes this relationship during water diuresis. Data obtained in 11 experiments on 2 dogs.

Pituitrin P was used as a preparation containing antidiuretic hypophiseal hormone; it was injected intravenously in the dose of 0.005 units per 1 kg body weight. In the first series of experiments the effect of pituitrin was studied with administration of the preparation after 3% water loading against the background of diuresis of such extent that no hyaluronidase activity could be detected in the urine. It was found that following pituitrin injection the diminution in diuresis was accompanied by a definite increase in hyaluronidase activity of urine, the activity changing in accordance with the magnitude of urinary excretion and coinciding exactly with the typical curve obtained in control experiments.

TABLE 1

Influence of Hypophyseal Antidiuretic Hormone and of In Vitro Concentration on the Hyaluronidase Activity of Urine

Original sample		Pituitrin administration		Activity of urine concentrated in vitro
diuresis	activity	diuresis in ml/min · m²	activity	
0.82	0	0.21	12	0
0.82	0	0.36	7	0
0.83	0	0.40	15	0
0.83	0	0.45	13	0
0.83	0	0.33	14	0
0.94	0	0.7	9	0
0.94	0	0.56	11	0
0.94	0	0.23	12	0
0.98	0	0.26	13	0
0.98	0	0.55	4	0
1.6	0	0.36	12	0
1.6	0	0.6	7	0
1.6	0	0.7	9	0

Special experiments established convincingly that the antidiuretic hormone exerted no effect on hyaluronidase in vitro. Consequently, the increase in hyaluronidase activity of urine following introduction of pituitrin cannot be the result of direct hormonal influence on the activity of the enzyme.

Increase in urinary activity after injection of pituitrin cannot be explained by a simple increase in hyaluronidase concentration during small diuresis either; this is clearly demonstrated by experiments with parallel investigation of urine concentrated in vitro under vacuum and in vivo as the result of antidiuretic hormone action.

The experimental procedure of appropriate experiments, whose data are presented in Table 1, consisted of the following: 2 samples of urine were collected from the animal, during extensive diuresis after hydration and after administration of pituitrin when the diuresis was considerably reduced. Part of the urine obtained during extensive diuresis was concentrated under vacuum at a temperature of 37° to a degree corresponding to the diminution of diuresis under the influence of pituitrin as compared with the original. The hyaluronidase activity was determined in all 3 samples.

As Table 1 shows, considerable increase in activity is found in the urine obtained after injection of pituitrin, whereas urine of the same volume but concentrated in vitro possesss no hyaluronidase activity. It follows therefore that increase of hyaluronidase activity under the influence of the anitdiuretic hormone is not determined by concentration changes and is, evidently, the result of increased liberation of the enzyme by renal tissue elements.

TABLE 2

Effect of Water-Osmotic Load and Pituitrin on the Hyaluronidase Activity of Urine

Water load		Administration of urea		Administration of pituitrin	
diuresis in ml/min · m^2	activity	diuresis in ml/min · m^2	activity	diuresis in ml/min · m^2	activity
Experiments on the dog Molli					
0.98	14	3.6	14	2.9	44
4.5	0	6.0	0	2.8	30
2.8	0	6.0	0	5.5	28
2.8	0	6.0	0	2.8	31
3.0	2	12.0	2	7.0	13
Experiments on the dog Kutsei					
1.2	0	2.5	0	1.9	11
1.8	0	7.0	0	1.1	11
1.5	0	5.5	0	2.2	13
4.5	0	11.0	0	2.8	16

However, in the experiments described, the effect of the antidiuretic hormone was investigated against the background of considerable reduction of diuresis when the urine usually shows hyaluronidase activity. In order to trace the action of antidiuretic hormone without appreciable fall in diuresis a combined water-osmotic load was used. The animal was given urea in the dose of 0.5 per 1 kg body weight against the background of developed water diuresis. When the diuresis increased still further the animal was given pituitrin intravenously.

The hyaluronidase activity was determined in the urine which was preliminarily dialyzed and adjusted to the volume corresponding to a diuresis of 0.2 ml/min × m^2 to exclude in all samples the possibility of salt or concentration effect.

As can be seen from Table 2, in which the data of this series of experiments is presented, water loading increases diuresis to such an extent that the urine either loses its hyaluronidase activity completely or only exhibits slight activity.

Administration of urea after water loading increases diuresis but exerts no influence on the magnitude of enzyme activity, since under conditions of hyperhydration of the organism the facultative reabsorption does not rise. Following injection of pituitrin, which increases distal reabsorption of water, the diuresis diminishes somewhat but thanks to the osmotic activity of urea, continues to maintain a high level considerably in excess of that during the initial water diuresis. Despite this, the urine acquires considerable hyaluronidase activity.

The experiments carried out suggest the following conclusions:

1. The dependence of hyaluronidase activity of urine on the extent of diuresis cannot be explained in terms of simple dilution, salt effect or change in the volume of urine in which the enzyme is distributed. The characteristic curve of hyaluronidase activity of urine depends on the differences in intensity of liberation of the enzyme by renal tissue elements associated with changes in the extent of urinary excretion.

2. The urine shows high hyaluronidase activity when the animal's blood stream has a high concentration of the antidiuretic hormone and when distal reabsorption of water is intense. Reduction in concentration of the antidiuretic hormone and of distal reabsorption of water, as is the case after water loading, is accompanied by a corresponding change in the enzyme activity of urine.

3. Liberation of the hyaluronidase enzyme by renal tissue elements is related directly to the process of distal reabsorption of water and is regulated by the antidiuretic hormone of the pituitary.

SUMMARY

The activity of hyaluronidase which was detected in the urine of man and animals in weak diuresis is changed according to a characteristic curve when urine excretion is increased. Dependence of its activity on the diuresis cannot be explained by dilution, salt effect or by the change of the volume of urine in which the enzyme is distributed. Evidently the curve of hyaluronidase activity is connected with varying intensity of liberation of the enzyme by the elements of kidney tissue.

High hyaluronidase activity is found in cases when the concentration of the antidiuretic hormone in the blood is high and intense reabsorption of water takes place in the distal segment of the nephron. This suggests that excretion of hyaluronidase by the elements of kidney tissue has a direct relationship to the process of active reabsorption of water. It is regulated by the antidiuretic hypophyseal hormone.

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THE ROLE OF BARORECEPTIVE SYSTEMS IN THE NASAL CAVITY OF BIRDS IN REGULATION OF RESPIRATION

COMMUNICATION II

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Our investigations into the effect of the baroreceptor olfactory system on respiration have been aimed at discovering role of this function in regulation of respiration at various stages of phylogenesis and its dependence on the animal's environment.

The first communication [1] described the role of olfactory nerve baroreceptors in the regulation of respiration in frog and the place occupied by this system among other baroreceptor systems situated along the respiratory passages. It seemed particularly interesting to study the baroreceptor system in the mucosa of the upper respiratory passages in birds who experience the effect of large currents of air exerting considerable pressure on the mucosa of the nasal activity. No references to this question could be found in available literature. Yet marked pressure on the receptor endings of nerves innervating the intranasal mucosa by air currents during flight must during the process of phylogenesis, have led to the appearance in birds of special mechanisms concerned with adaptation of the intranasal different system to receiving strong baroreceptor stimuli.

The present work is concerned with the study of the role of olfactory nerve baroreceptors in the regulation of respiration in birds as well as of compensatory, adaptational mechanisms in the central nervous system.

EXPERIMENTAL METHODS

A total of 30 experiments was performed on 21 pigeons. The weight of the birds varied from 525 to 670 g. Respiratory movements were recorded by a modification of N. G. Goleva's method [2] using a rubber cuff placed around the chest and abdomen of the pigeon and connected to a Marey's tambour. The cuff was slightly inflated with air before the experiment. Stimulation of the intranasal mucosa baroreceptors was achieved by rhythmic introduction of a stream of air into the mask placed over the bird's beak. The pressure and rhythm of this air stream was recorded on a kymograph. All the experiments were carried out on tracheotomized pigeons with transected esophagus in order to exclude mechanical stimulation of the upper portions of the gastro-intestinal tract by the applied air stream.

Olfactory afferents were excluded by means of extirpation of the olfactory lobes and the trigeminal afferents were excluded by painting the intranasal mucosa with a 2% solution of cocaine. In order to demonstrate the relationship between the cerebral cortex and the subcortical formations of the intranasal baroreceptor system either the cerebral hemispheres were removed or the cerebral cortex was stimulated by application to it of 0.1% solution of strychnine.

EXPERIMENTAL RESULTS

Initial respiratory rate in pigeons was from 45 to 60 per minute, the amplitude of the movements being from 4 to 10 mm. Following tracheotomy the rate of respiratory movements dropped to 24-40 per minute and

the amplitude fell to from 1 to 7 mm. The form of the movements was unchanged but the general background of the respiratory trace became considerably more even.

In most cases there was no respiratory reaction to application of a stream of air to the nasal passages of tracheotomized birds even when this was done under high pressure (up to 180 mm water). Only in 6 cases was a respiratory reaction observed; it consisted of a slight increase in the rate of undulation of the background respiratory trace without change in the amplitude of the respiratory movements (Figs. 1 and 2).

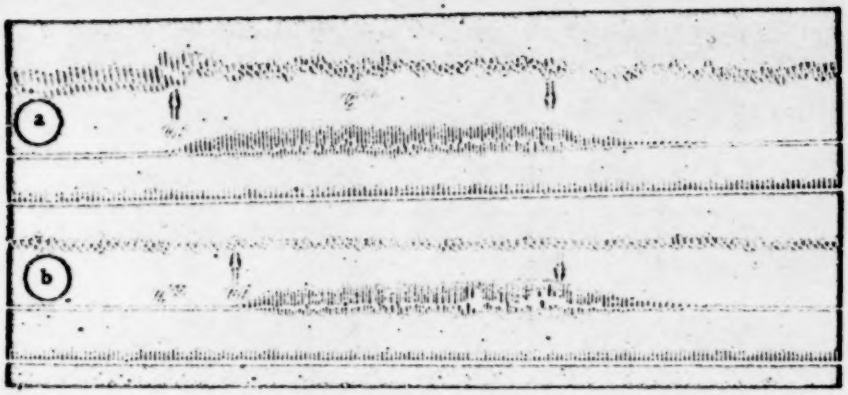

Fig. 1. Effect of tracheotomy on the character of respiration and on respiratory reaction in pigeon.
a) Initial respiration; b) respiration and absence of respiratory reaction to insufflation of air following tracheotomy (↑ beginning, ↓ cessation of insufflation). Records from above down: pneumogram, record of air insufflation; time marker (1 second).

Extirpation of the olfactory bulbs had little effect on respiration. In most experiments the respiratory movements showed some increase in rate (from 30 to 33-36 per minute) and increase in their amplitude (from 4-7 to 6-10 mm).

Most birds showed absence of respiratory reaction to insufflation of air into the nasal passages following removal of the olfactory lobes.

The experiments revealed that under normal conditions in the case of birds, unlike that of frogs, baroreceptor stimulation of the intranasal mucosa caused almost no changes in respiration even when high pressures were used. This can be explained by the hypothesis that the environment, i.e., almost constant influence of strong air currents on the nasal baroreceptor system during flight caused the appearance, in the process of phylogenesis in pigeons, of some compensatory mechanisms adapting the activity of this system to various conditions of existence. In order to discover these adaptive mechanisms the pigeons were subjected to removal of various parts of the central nervous system, including extirpation of the cerebral hemispheres.

Removal of the cerebral hemispheres in pigeons, performed in 9 experiments, caused an increase in respiratory rate in 6. Changes in the amplitude of respirations were not characteristic: in 4 experiments there was a decrease in amplitude, in 4 an increase and in one it remained unaltered. It must be noted that there was a change in the form of respiratory movements as well, as they were stepped up

It is significant that following extirpation of the cerebral hemispheres in pigeons there was an increase in the respiratory reaction to rhythmic stimulation (even under small pressure – 30-60 mm water) of the intranasal baroreceptors. These reactions were expressed in reduction of the rate and amplitude of respiratory movements; this phenomenon was so marked in a number of experiments that apnea occurred either in expiration or in inspiration and persisted throughout the period of stimulation (Figs. 2 and 3).

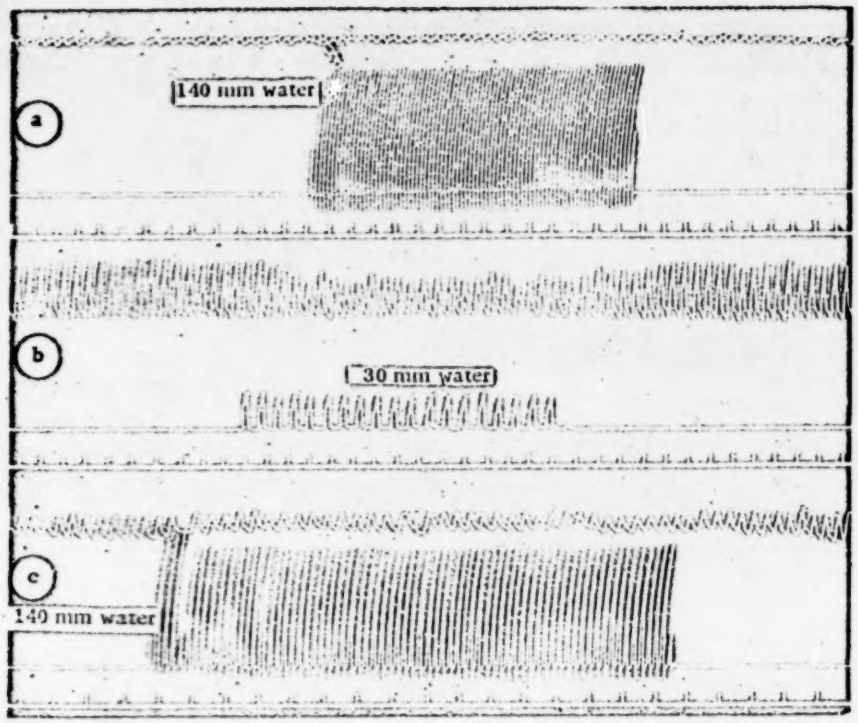

Fig. 2. Effect of extirpation of the cerebral hemispheres and subsequent removal of olfactory lobes on respiration and respiratory reaction in pigeon.
a) Respiration and respiratory reaction to insufflation of air after tracheotomy; b) respiration and respiratory reaction after extirpation of cerebral hemispheres; c) the same after removal of olfactory lobes. Records from above down: pneumogram, record of air insufflation; time marker (5 seconds).

These experiments revealed clearly the role of the cerebral hemispheres in the regulatory mechanism adapting respiration to various afferent impulses from the intranasal baroreceptor system. These data find confirmation also in experiments with stimulation of the cerebral cortex by strychnine (piece of filter paper 4 × 4 mm^2 moistened with 0.1% solution of strychnine); in these experiments even strong baroreceptive stimulation of the intranasal mucosa (above 180 mm H_2O) produced no respiratory reaction. The inhibitory and corrective influence of the cerebral hemispheres on the subcortical parts of the respiratory center has thus been established.

Removal of the olfactory lobes in birds deprived of their cerebral hemispheres (6 experiments) caused reduction of frequency and amplitude of respiratory movements. Respiratory reaction in response to rhythmic stimulation by an air stream was absent in the majority of experiments or only appeared in the form of a slight reduction in the amplitude of respiratory movements without a change in rate.

The removal of the olfactory baroreceptor system thus abolished or reduced the excitability of the intranasal mucosa with respect to stimuli produced by air pressure. It can be concluded on the basis of the data presented that the olfactory nerve baroreceptors in birds from part of an afferent baroreceptor system of the upper respiratory tract which exerts an influence on the respiratory center. The reactivity of the whole system is related to the conditions of the animal's environment. The highest reactivity was encountered in land or amphibious animals (frogs, lizards, rabbits, dogs). Under such conditions of environment even small fluctuations

Fig. 3. Effect of extirpation of cerebral hemispheres on the respiratory reaction in pigeon.
a) Prior to extirpation of cerebral hemispheres; b) after removal of cerebral hemispheres. Records from above down: record of air insufflation, pneumogram, time marker (5 seconds).

in air pressure within the nasal cavity signal considerable changes in the environment [4]. The quite different conditions of existence in the case of birds lead to the development of a regulatory apparatus which suppresses the effect of large fluctuations of air pressure on the intranasal mucosa. Such a regulatory influence is exerted by the cerebral hemispheres which, according to our data, lower the excitability of the whole afferent baroreceptor system of the nasal cavity of birds during flight.

SUMMARY

The author demonstrated the existence of afferent baroreceptive olfactory-respiratory system in birds.

The function of this system in normal conditions is depressed by the cerebral cortex and is manifested only after the removal of the latter.

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REDISTRIBUTION OF THE BLOOD IN THE BLOOD VESSELS OF LOWER MONKEYS

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The morphologic composition of the blood vessels is known to be different [2, 5, 6, 8, 9]. According to the data of the majority of investigators the number of leucocytes in the visceral blood vessels is lower than that in the blood vessels of fingers, ear lobe and skin. Such a migration of leucocytes within the body from one vascular area to another has received the name of "distribution leucocytosis."

At the same time some research workers deny the possibility of redistribution of blood in the organism [7]. In their view, if there is a difference in the values of blood indices from one vascular area to another it is connected not with redistribution of the blood but with a generally higher content of leucocytes in the skin as compared with large blood vessels and peripheral veins (since "peripheral blood" often implies capillary blood taken by puncturing the skin). However, this view requires a more thorough approach to its verification.

Literature references to the composition of the blood of internal blood vessels (veins and arteries) are limited chiefly to data on the total number of leucocytes, the question of the amount of hemoglobin and erythrocytes being covered inadequately.

The present investigation was carried out on lower monkeys of various species (Macacus rhesus, baboon Papio tramadryas, marmoset and gelada).

EXPERIMENTAL METHODS

Ten adult males were used in the experiments. The monkeys were kept on similar diets and under identical conditions. Blood samples were taken twice a week at 8-9 a.m. before the animals were fed. Composition of the peripheral blood (erythrocytes, leucocytes, hemoglobin and differential leucocyte count) was determined in capillary blood from the right ear; composition of venous blood was determined on specimens taken from the right cubital vein. The venous blood was first mixed with sodium citrate (powdered); after 1 minute cell counts were made and the amount of hemoglobin determined (by the Sahli method). For the sake of convenience of presentation the hemoglobin indices are given as percentages. All specimens and the smear were treated in the usual manner. The elapsed time between the collection of blood from the ear and from the vein was no greater then 5-7 minutes. The blood was usually collected by the same persons under standard conditions of experimental environment over a period of 7-17 days.

EXPERTIMENTAL RESULTS

Red cells. The number of erythrocytes in 1 mm^3 blood taken from the vein is 20-50% lower (average) in monkeys than in blood taken from the peripheral blood vessels of the ear (see Table).

The Table shows that venous blood contains less hemoglobin than peripheral blood. The two show parallel changes in these indices.

TABLE

Average Values for Composition of Blood Obtained from the Ear and from a Vein in Lower Monkeys

Composition of the blood	Monkeys																			
	Bering		Erdzhis		Artam		Azon		Zher		Pion		Beglets		Slepen		Kipr		Klaro	
	ear	vein	ear	vein	ear	vein	ear	vein	ear	vein	ear	vein	ear	vein	ear	vein	ear	vein	ear	vein
Erythrocytes (in millions)	6.027	4.222	5.368	4.696	5.315	4.378	4.550	4.160	3.652	3.605	4.992	4.385	5.678	4.393	4.366	4.346	—	—	—	—
Hemoglobin (in %)	112.7	83.10	105.0	98.0	84.2	72.1	80.0	78.0	80 27	71.5	87,5	77.5	100	85	74.6	70.6	—	—	—	—
Leucocytes (in thousands)	12.5	7.31	11.4	5.2	11.2	7.6	16.0	11.0	14.2	10.2	13.1	8.4	16.3	10.07	12.7	6.9	10.6	7,3	12.7	6.9
Polymorphonuclear neutrophils (in absolute numbers)	2045.4	1490.5	3351	2615	3213,1	3082,3	5206.4	4658.4	6263.4	4271.4	6881.8	4343.7	3322.6	2204,5	4342.5	3251.5	6861.0	5773.0	3976.8	2682.0
Lymphocytes (in absolute numbers)	8240.5	4679 0	6041	4160.5	6695.5	3881.7	8641.2	5505.6	6224.0	4030	5368.7	2517.3	10 042.4	6284.2	6457	3563.0	2404.2	1501.0	6658.6	3544.8
Monocytes (in absolute numbers)	429.4	237.1	621.5	286.5	371.1	154.0	612 8	548.8	649.7	449,0	485.2	256.0	1274.8	523.4	711	175.5	350.2	194.0	618.0	245.0
Basophils (in absolute numbers)	—	—	—	—	—	—	—	—	—	—	66.0	9.0	246	63,6	—	—	—	—	167,6	32.4
Eosinophils (in absolute numbers)	1209.7	657.1	181.5	64.5	126.2	250.5	649.6	352,6	330	365.4	495.2	135.0	1348.6	663.0	434	262.5	—	—	559,6	273.2
Color index	0.93	0.98	0.99	1.06	0,79	0.83	0.88	0,95	1.05	0.98	0,95	0,89	0,89	0,98	0.86	0.81	—	—	—	—

In the case of the monkey Bering, for example, the difference in the number of erythrocytes in venous blood and in capillary blood from the ear (average of 5 estimations) is 1,805,000 and the difference between the hemoglobin values in venous blood and in blood from the ear is 29.6%. In the case of the monkey Artam the former difference is 937,000, the latter 12.1%; in the case of Azon the differences are 390,000 and 2% respectively. The difference in hemoglobin content in different blood vessels is thus determined by the number of erythrocytes.

White cells. The number of leucocytes in venous blood is lower than in blood taken from the ear; this was found in all the monkeys examined (see Table). The difference in individual cases reached approximately 50-70%.

It must be noted that the number of leucocytes in peripheral blood of the monkeys was usually higher during the first few days than later on. Repeated examinations of the blood under identical experimental conditions lead to considerable lowering of the total number of leucocytes per 1 mm^3 of blood. Thus, in the case of the monkey Pion the average number of leucocytes determined in the first 5 analyses was 15,370 and 12,560 according to the last 5 analyses; in the case of Kipr the numbers were 12,560 and 8,760 respectively, in the case of Beglets 18,600 and 14,400 respectively. This phenomenon has been described by I. A. Utkin and M. I. Kuksova [3] and was regarded as extinction of reaction to experimental environment. This extinction phenomenon is also shown by the total number of leucocytes in venous blood. For example, in the case of the monkey Kipr the average number of leucocytes in venous blood was 9,040 according to the first 4 analyses and 7,360 according to the last five; in the case of Beglets the values were 10,100 and 9,300 respectively. This tendency was less pronounced in the monkeys Zher and Erdzhis.

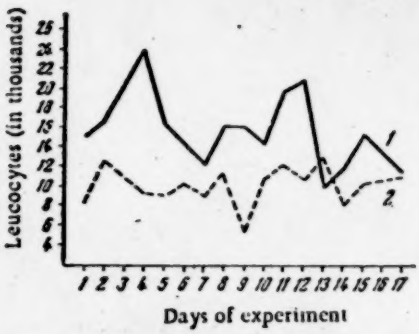

Fig. 1. Changes in the total number of leucocytes in venous blood and blood taken from ear capillary in the monkey Beglets during repeat examinations over a period of several days.
1) Number of leucocytes in the peripheral blood vessels of the ear; 2) number of leucocytes in the cubital vein.

The greatest difference between the total number of leucocytes in blood taken from the vein and from the capillary of the ear was observed during the first days of the investigation when the animal's reaction to experimental environment was more pronounced. This is expressed in increased leucocyte content predominantly in peripheral blood. On extinction of the reaction to environment the total leucocyte values in both cases tend to approach each other as the result of a definite drop in their number in the blood obtained from the ear (Figs. 1 and 2).

On some days the number of leucocytes in venous blood exceeds that of peripheral blood but, as a rule this difference is very slight and occurs after extinction of changes in peripheral blood elicited by reaction to the environment.

A differential leucocyte count (in absolute numbers) revealed that venous blood contained fewer lymphocytes, neutrophils, monocytes and eosinophils than peripheral blood.

The ratio of the main cells of the white series – lymphocytes and polymorphonuclear neutrophils, characteristic for each species of animal as determined from peripheral blood, is maintained also in venous blood (Fig. 3).

The material presented in this paper suggests that the dissimilar content of cellular elements of the blood and of hemoglobin in different blood vessels of the body is a consequence of redistribution over various areas of the vascular system. The differences in the composition of venous and peripheral blood are not qualitative but only quantitative in character.

The process of blood redistribution in the body is achieved by the neurohumoral route with participation of the higher sections of the central nervous system [1] and is related to "true" distribution of the blood associated with bone marrow activity.

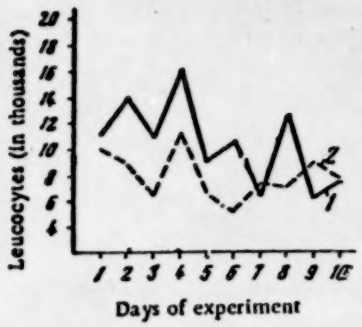

Fig. 2. Changes in the total number of leucocytes in the blood collected from the ear capillaries and a vein in the monkey Kipr during repeat examinations over a period of several days. Legend the same as in Fig. 1.

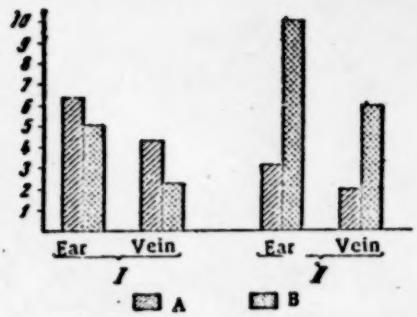

Fig. 3. Ratio of polymorphonuclear neutrophils (A) and lymphocytes (B) in ear capillaries and in a vein of the monkeys Pion (I) and Beglets (II).

In the light of our data it is tempting to consider the work of A. I. Chukavina [4] with respect to "local" and "general" leucocytosis. This author failed to obtain a significant difference in healthy subjects in the number of leucocytes obtained from the skin of various regions of the thorax ("local" leucocytosis) and from the finger ("general" leucocytosis). Nor were any differences in the numbers of leucocytes at the same sites noted after subcutaneous administration of atropine, pantopon and nitroglycerin. This should have been expected, since determination of "local" and "general" leucocytosis in the given case was carried out on peripheral areas of blood vessels. The skin of the fingers and of the chest or abdomen is essentially a peripheral part of the body and therefore regardless of the skin site of blood collection, the numbers of leucocytes found will, it seems to us, be relatively similar.

SUMMARY

Experiments were performed on various species of monkeys. It was established there was a decreased number of erythrocytes, leucocytes (of the total number, as well as of their separate forms) and hemoglobin in the blood taken from the vein in comparison with the blood taken from capillaries of the ear lobe. The color index (which reflects the degree of saturation of a single erythrocyte by hemoglobin) and the ratio of the lymphocytes and neutrophils are the same in both specimens of the blood. The difference between the total number of leucocytes in the ear capillary and in the vein is greatest during the first few days when the animal is not yet used to the conditions in which the experiment is performed. With a decline of the reaction to the environmental conditions, the difference between the total number of leucocytes taken from the ear capillary and the vein becomes less pronounced due to the decrease of leucocytes in the blood taken from the ear. This demonstrates that uneven distribution of the cellular elements and hemoglobin in various vessels of the organism is the result of redistribution of the blood.

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THE EFFECT OF VITAMIN C ON THE ABSORPTION OF GLYCINE AND CHLORIDES BY THE INTESTINE

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Vitamins play a direct part in the absorption of foodstuffs by the gastrointestinal tract. Much work has been devoted to the study of the role of vitamins in the regulation of absorption but the problem still remains inadequately solved.

Mottram, Cramer, Drew [5] noted stimulation of glucose and peptone absorption under the influence of the vitamin B complex. Analogous data have been obtained by Verzar [9] and S. R. Perepelkin [3].

Schraeder [7] has observed stimulation of glucose absorption under the influence of vitamin C.

E. I. Kleitman (1938) found that experimentally produced vitamin C deficiency did not affect fat absorption but if the diet were made deficient in vitamins B, A and D there was an adverse effect on absorption of fats and glucose.

Vogel [10] notes that vitamin B, if given in extremely large doses, accelerates glucose absorption.

We have shown in previous long-term investigations [1] on dogs, the conditions necessary for absorption of vitamin C and its effect on the absorption of glucose in the small and large intestine and in the lesser sac of the greater curvature. Part of the data concerning the influence of the vitamin on absorption of glucose in the small intestine was obtained from clinical observations on patients in hospital.

Since the question of the influence of vitamin C on the absorption of glycine and chlorides has not been sufficiently elucidated we undertook to study the role played by vitamin C in the regulation of glycine absorption in the small intestine and of chloride absorption in the colon.

EXPERIMENTAL METHOD

The present communication deals with experimental data obtained in experiments on 3 dogs – Dzhek, Tuz and Geroi – with chronic fistulae of the small and large intestine. Experiments were performed 12-14 hours after feeding. The solution being investigated (8 ml) was introduced into the isolated intestinal loop; after 5 minutes it was allowed to run into a measuring cylinder and the intestinal loop was immediately washed out. The washings were also carefully collected and measured. The amount of the test substance absorbed was determined by the amount of it found in the collected part of the solution and the washings. In experiments on the small intestine 0.75% solution of glycine and 0.05% solution of ascorbic acid were used; in experiments on the large intestine the solutions used were 0.85% sodium chloride and 0.05% ascorbic acid. The dissolved vitamin was added to the solution of the test substance so as to obtain definite ratios by volume such as 4 : 1, 3 : 2, 2 : 2 or 4 parts glycine solution to one part vitamin solution etc. If vitamin C was added in the solid state the ratio of glycine to vitamin was maintained. In control experiments water was added instead of the vitamin in the same proportions by volume. The glycine content of the fluids examined was determined by

formol titration. Chlorides were estimated by potentiometric titration, vitamin C by a simplified version of the Lavrov method.

EXPERIMENTAL RESULTS

Experimental data showed that in the case of the dog Dzhek (Table 1) 0.05% solution of ascorbic acid was absorbed to an average extent of 42% during the 5 minutes that it was present in the intestinal loop; absorption of glycine during the same period of time amounted to 40%.

When vitamin C was added to a solution of glycine in various proportions it was found that the optimal ratio for glycine absorption was 2 : 2 when the absorption of glycine increased by 28% as compared with the control. Addition of vitamin C to a solution of glycine in the ratio of 3 : 2 also increased glycine absorption in the intestine (by 24%). When the ratio was 4 parts of glycine to 1 part of the vitamin no improvement in glycine absorption was noted.

TABLE 1

Influence of Vitamin C on Absorption of Glycine in the Small Intestine in Dogs (Average Values in Percentages)

Dog	Time (in minutes)	Absorption of 0.75% glycine solution	Absorption of 0.75% vitamin C solution	Glycine + vitamin C		
				4 : 1	3 : 2	2 : 2
Dzhek	5	40	42	41	64	68
Tuz	5	56	54	65	82	60

Experiments on the dog Tuz (Table 1) showed that glycine was absorbed from 0.75% solution to the extent of 56% during 5 minutes and vitamin C from 0.05% solution to the extent of 54%. Addition of vitamin C to the glycine solution enhanced the absorption of the latter both when the ratio was 3 : 2 and when the value differed, but the effect was more marked in the former case.

As already mentioned, the vitamin was added to the glycine solution in the form of a solution in some experiments and in the solid state in others when it was calculated to give the same ratios of glycine to vitamin. In both cases the results were similar, i.e., absorption of glycine was increased to the greatest extent when the ratio of glycine solution to vitamin was 3 : 2 in the case of Tuz and 2 : 2 in the case of Dzhek.

In order to confirm that glycine absorption was improved by vitamin C and not simply by dilution of the glycine solution with water, control experiments were staged in which water in the same proportions was added instead of the vitamin (Table 2).

It follows from Table 2 that when the vitamin was replaced by water in the same proportions the absorption of glycine not only did not improve but in some cases showed a decrease.

The next section of the work was devoted to the study of the influence of vitamin C on the absorption of chlorides in the colon.

In work dealing with the absorption of chlorides much attention is usually paid to various factors affecting this process. Blickenstaff and others [4] note that chloride absorption is affected by the pressure within the intestinal lumen. References to the dependence of the rate of chloride absorption on the concentration of the solution being absorbed are encountered in the work of Verzar [10], Starling [9], Wells [12], Rabinovich [6]. According to L. P. Pankova [2] the absorption of chlorides is influenced by the presence in the intestinal lumen of intestinal juice, pancreatic juice and bile.

These references to data available in the literature indicate that no direct investigations have been made into the influence of vitamin C on the absorption of chlorides.

TABLE 2

Absorption on Addition of Vitamin C Solution and of Water (Average Data in Percentages)

Dog	Absorption of 0.75% glycine solution	Volume ratio of 0.75% glycine solution to vitamin solution and to water					
		glycine + vitamin C	glycine + water	glycine + vitamin C	glycine + water	glycine + vitamin C	glycine + water
Dzhek	40	–	–	64	47	68	49
Tuz	56	–	–	82	50	60	37

Data in Table 3 show that sodium chloride in 0.85% solution was absorbed to the extent of 13% during the 5 minutes that it was in the isolated intestinal loop and ascorbic acid in 0.05% solution was absorbed to the extent of 17.8% during the same period of time.

Addition of ascorbic acid in the solid state to the sodium chloride solution in the ratio of 4:1 decreased the absorption by half.

TABLE 3

Influence of Vitamin C on Absorption of 0.85% Sodium Chloride Solution in the Colon in the Dog Geroi During a Period of 5 Minutes (Vitamin C Added in the Solid State)

Solution	Absorption (average values in percentages)
0.85% sodium chloride solution	13.0
0.05% ascorbic acid solution	17.8
4:1 solution of sodium chloride + ascorbic acid	6.5
3:2 sodium chloride solution	23.0
2:2 ascorbic acid	27.0

Increasing the concentration of the vitamin led to increased absorption of chlorides in the colon. For example, with a ratio of sodium chloride and solid vitamin C 3:2 the absorption increased to 23%. When solid vitamin C was replaced by its solutions with preservation of the proportion of sodium chloride to the vitamin, a similar picture of absorption was observed: chloride absorption was enhanced to the greatest extent when the ratio was 3 parts sodium chloride to 2 parts vitamin C (48% as against 13% in the control).

Vitamin C in certain doses thus enhances the absorption of glycine in the small intestine and of sodium chloride in the colon.

SUMMARY

The effect of ascorbic acid on the regulation of absorption of glycine was studied in experiments on 3 dogs with chronic fistulae of the intestine and colon. It was established that ascorbic acid, administered in definite doses, improved absorption of glycine in the intestine and of sodium chloride in the colon. 3:2 and 2:2 are the optimal ratios of glycine and sodium chloride to the vitamin. In the presence of the above ratio absorption of glycine in the intestines increases by 24-28%, while absorption of chlorides in the colon increases by 18%.

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TONIC REFLEXES IN MAN

COMMUNICATION II. EFFECT OF UNSTABLE POSITION OF THE BODY ON THE A. A. UKHTOMSKII TONIC REFLEX

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It is known that muscle tone which is based on tonic reflexes ensures adaptational reactions of the human body directed towards counteracting the force of gravity and maintenance of an erect posture.

In the present work an attempt was made to trace the influence of an unstable position of the human body on muscle tone; the proprioceptive tonic reflex described by A. A. Ukhtomskii in 1925 [1] and studied by other workers [2, 3, 4, 5] was chosen as an experimental tool.

EXPERIMENTAL METHOD

In studying the effect of an unstable position on the extent of change in the tone of skeletal muscles the magnitude of tonic reflexes was first determined with the subject in a stable position; the subject's body was then placed in an unstable position. A total of 53 experiments was carried out on students of the Institute of Physical Culture.

The stable position of the body was changed in the following ways.

1. A stable position was achieved by standing on skates in a special wooden stand with the weight resting on the whole sole of the boot. After recording the reflex to a push in the stable position, the same reflex was recorded with the subject standing on skates on a smooth glass surface, i.e., in an unstable position.

2. During the experiment the subject was standing on a hard surface over which a sheet of rubber was stretched. On termination of the repulsion the hard surface was lowered by pressing a lever and the subject descended together with the rubber sheet (within the limits of its elasticity), passing suddenly into an unstable position at the moment of onset of the Ukhtomskii reflex.

The myotopographic technique was used for recording the repulsion and the reflex response.

EXPERIMENTAL RESULTS

The data yeilded by the first method showed that on passing from a stable position of the body to an unstable one the tone of extensor muscles of the extremities was increased. This was particularly marked in the case of subjects who had never before stood on skates.

When the body was in an unstable position during repulsion the tonic reflexes were considerably enhanced. Thus, when the reflex displayed by the subject V. G. while standing in the special stand with the usual area of support is compared with that displayed while standing on skates it is seen that the raising of the arm increased by 40° (Fig. 1).

A sudden displacement from a stable position prior to repulsion is associated with an involuntary raising of both arms which is represented on the myogram by a vertical rise in the trace (Fig. 2). If, however, the subject is first pushed away from vertical support then the displacement from a stable position at the moment of onset of the reflex produces considerable increase and prolongation of the tonic reflex (raising of the arm increased by 30-40°). Those subjects who do not show a visible tonic reflex reveal increased tone in the deltoid muscle of the arm being repulsed when the supporting surface is suddenly lowered; this is expressed in the myogram (Fig. 2) by a tonic afterdischarge following the involuntary raising of the arms (vertical rise of the trace).

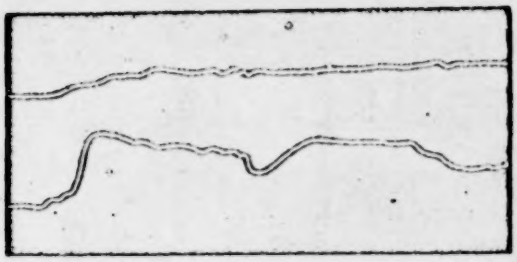

Fig. 1. Change in the tone of the deltoid muscle of the shoulder girdle while in an unstable position (standing on skates); subject V. G. Records from above down: myogram of the left deltoid muscle, myogram of the right deltoid muscle.

The difference in the changes of tone of the deltoid muscle during standing on skates and on solid surface is less in students of the Institute of Physical Culture than in untrained subjects; this is evidently due to the fact that the former have already developed motor skills with appropriate changes in muscle tension. An important part is played by regulation of skeletal muscle tone in the process of learning and training, with increasing perfection and precision with respect to proportioning tonic tension of the muscles involved in a given movement.

Fig. 2. Change in the tone of deltoid muscle of the shoulder on sudden displacement of the subject from a stable position. Records from above down: myogram of the left deltoid muscle, myogram of the right deltoid muscle; ↓moment of displacement from a stable position.

The proprioceptive tonic reflex described by A. A. Ukhtomskii can thus be used as one of the components of the whole system of tonic reflexes of skeletal muscles for the study of the mechanism of changes in muscle tone under conditions of unstable position of the body.

It may be concluded on the basis of the data obtained that an unstable position of the body or sudden displacement of the body from a stable position increases the tonic reflex. In cases in which this reflex was not previously visibly apparent, increased muscle tension was found in the arm pushing away when the body was displaced from the stable position.

SUMMARY

It was established that the tone of extensor muscles of the extremities is increased when the position of the body is made unstable. Experiments were performed on man. The change of the tonic condition of deltoid muscles was observed. The proprioceptive tonic A. A. Ukhtomskii reflex served as a model. The method of myotonography was used for recording.

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PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

SERUM ALDOLASE CONTENT OF THE BLOOD OF DOGS WITH EXPERIMENTAL (MYOCARDIAL) INFARCTION

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In recent years, the most actively pursued field of medical research has been that dealing with the problems of acute coronary insufficiency and of myocardial infarction. It appeared from the discussions held at the XIV Congress of Therapists that, despite the considerable advances achieved in the study of myocardial infarction, many aspects of the problem still await solution. Among these is the study of biochemical changes taking place in the myocardium during the course of this disease.

Recently published papers on myocardial infarction have drawn attention to the importance of changes in the myocardial enzyme systems, and in particular to the transaminases, which are responsible for transamination reactions. These researches have led to the provision of a new diagnostic method, permitting the assessment of the activity of the necrobiotic process [1, 5, 6].

It is known that considerable distrubances of the carbohydrate metabolism of the myocardium are encountered during infarction, glycolysis being affected in particular. There can be no doubt that the enzyme systems taking part in this process must undergo some changes. We have undertaken a special study of one of the enzymes concerned in glycolysis, viz., aldolase.

We have been unable to trace any Soviet work on changes in aldolase activity in myocardial infarction; only quite recently have we found any references to changes associated with acute coronary insufficiency in the foreign literature [7]. Since we considered that this question has been insufficiently elucidated, we undertook an investigation into changes in aldolase content in myocardial infarction, and into the possibility of applying our findings for diagnostic and prognostic purposes.

O. Meyerhof and K. Lohmann [8], in 1934, discovered a glycolytic enzyme, which they named zymohexase, responsible for catalyzing the breakdown of fructose 1:6-diphosphate into two triose phosphate molecules (one molecule of glyceraldehyde 3-phosphate and one molecule of dihydroxyacetone phosphate). This enzyme was later called aldolase.

In 1943 O. Warburg and W. Christian [9] were able to isolate this enzyme from tissues, in a crystalline form, and to establish the importance of its role in tissue glycolytic processes.

Subsequent papers have shown that changes in the content of this enzyme in the blood serum and tissues are encountered in various conditions involving tissue destruction and necrosis, such as tumors [9], liver disease [10], and viral diseases [4]. The highest aldolase contents are found in skeletal muscle, heart muscle, and liver [11], for which reason the widest variations in its content were found in diseases of these tissues.

It is noteworthy that in severe myodystrophic conditions there is a pronounced fall in muscle aldolase content, with a simultaneous rise in serum aldolase [5].

In view of the above considerations raised levels of serum aldolase might be expected to occur in

myocardial infarction, involving marked necrotic and dystrophic changes in the myocardium. Since the rise in serum aldolase content is associated with its liberation from injured cells, there should be a certain correlation between the degree of myocardial infarction and the rise in serum aldolase. These were the problems which we hoped to clarify in our research. Our investigations were performed on both experimental clinical material.

The present communication deals only with the experimental part of our studies, on the aldolase content of blood serum at various times after induction of myocardial infarction in dogs.

EXPERIMENTAL METHODS

Myocardial infarction was induced by ligation of the left coronary artery of dogs. This operation leads to the appearance of severe necrotic lesions of the anterior wall of the left ventricle, in the interventricular septum, and at the apex of the heart [2].

We ligated the left coronary artery of 16 dogs, of which two died within 20-25 minutes of ligation. Serum aldolase levels were determined for the surviving 14 dogs, at various times after the operation. Since the operation itself was associated with considerable skeletal muscle trauma, and with possible liberation of aldolase therefrom, we also determined the serum aldolase of 5 dogs which had been subjected to thoracotomy alone, without coronary ligation.

Electrocardiographic examination of all the operated dogs gave evidence of changes characteristic of myocardial infarct: negative T_1 and T_2 waves, displacement of the RS-T interval, accentuated Q wave. Autopsy of 3 dogs which died some time after ligation showed profound necrotic changes in the anterior wall of the left ventricle.

Aldolase was determined by the method of Dounce and Thannhauser, as described by V. I. Tovarnitskii and E. N. Valuiskaia.

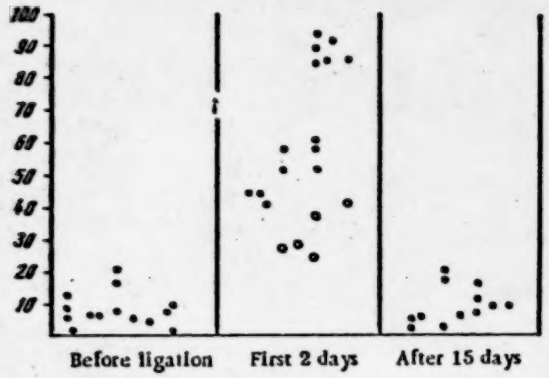

Fig. 1. Serum aldolase content of a dog subjected to experimental infarction and thoracotomy.

Aldolase was determined before ligation of the coronary artery, during the first 2 days after ligation, and then at intervals up to the 20th day.

A marked rise in serum aldolase was observed during the first 2 days after operation (Fig. 1). The aldolase content was in many cases 8-9 times higher than the initial level. In the control group (thoracotomy not followed by ligation) the rise in serum aldolase was much smaller, amounting to $2\frac{1}{2}$ to 4 times the initial level (represented by circles in Fig. 1).

We also observed a correlation between the size of the myocardial infarct and the rise in serum aldolase content.

Our findings are exemplified by 2 dogs,which died in a late stage of myocardial infarction. The postoperational condition of one dog was very grave, with severe symptoms of cardiac insufficiency, and with considerable electrocardiographic changes. Serum aldolase was 9 times higher than normal on the second day after the operation. The dog died on the 8th day. Autopsy revealed extensive infarction of the anterior wall of the left ventricle and of the apex and septum (Fig. 2). In the second dog, which died 21 days after ligation of the coronary artery, the myocardial infarction was much smaller, and was restricted to the anterior wall of the left ventricle (Fig. 3). The rise in serum aldolase was much smaller in this case (up to 58).

Fig. 2. Experimental myocardial infarction (↑) after high ligation of the left coronary artery.

These examples reveal a certain proportionality between the extent of the necrotic lesions of the heart wall and the level of serum aldolase. This finding can doubtless be applied for clinical purposes, both for diagnosis and prognosis.

In all the 14 cases observed by us we found a close correlation between the extent of the necrobiotic changes in the heart muscle and the increase in blood aldolase content.

In a number of experiments we compared the times of appearance of the biochemical changes in the blood after ligation of the coronary artery with those found after simple thoracotomy (control experiments).

We found the same rise in serum aldolase during the first few hours after ligation as after control thoracotomy, and this rise can most probably be ascribed to trauma of skeletal muscles associated with the operation. However, the aldolase content continued to rise 5 hours after ligation, and attained considerable proportions after 18 hours. Electrocardiograms taken at this stage did not always show the characteristic signs of myocardial infarction. This finding points to the possible diagnostic value of blood aldolase determinations in cases of suspected myocardial infarction.

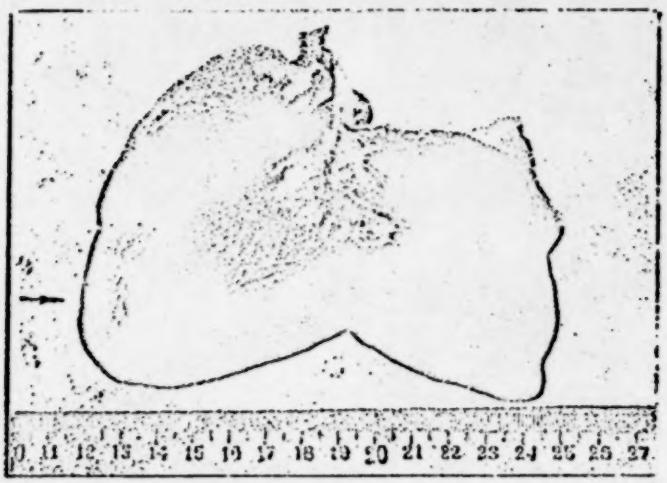

Fig. 3. Experimental myocardial infarction (→) after low ligation of the left coronary artery.

Blood aldolase levels began to fall on the 3rd-4th day, and were normal after the 15th day.

We determined blood aldolase in a series of 12 patients suffering from myocardial infarction, and in a series of 15 patients with "spurious" infarction, in whom the diagnosis of coronary thrombosis had been rejected on clinical grounds; our findings resembled those reported above for experimental animals.

SUMMARY

The content of aldolase in the blood serum was examined in dogs with experimental infarction induced by ligation of the left coronary artery. It was established that the content of aldolase is increased in these dogs by 4-5 times in comparison with that in the control animals. The concentration of the enzyme begins to increase 5-6 hours after the ligation of the coronary artery and reaches its maximum within the first 2 days. By the 15th day the content of aldolase returns to the normal level. The increase of aldolase concentration depends on the severity of myocardial infarction. Determination of the concentration of aldolase in the blood serum may be used for clinical diagnosis in suspected myocardial infarction.

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ROLE OF INTEROCEPTORS IN THE REGULATION OF OXYGEN SATURATION OF ARTERIAL BLOOD

COMMUNICATION III. EFFECTS OF DENERVATION OF THE CAROTID SINUS REGION ON OXYGEN SATURATION OF ARTERIAL BLOOD AND ON PULMONARY VENTILATION

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Our earlier observations [1], based on chronic experiments on animals, established a relationship between the degree and the dynamics of saturation of arterial blood with oxygen, under hypoxic conditions, and the function of the sinocarotid receptor zones. These findings permitted us to draw the conclusion that the dominating role is played in this process by the respiratory disturbances appearing after denervation of the carotid sinuses.

The object of the present research was to investigate the dynamics of saturation of arterial blood with oxygen, and to follow pulmonary ventilation changes after denervation of the carotid sinuses. As controls we used observations of oxygen saturation of blood after denervation of the spleen and after division of the femoral nerves.

EXPERIMENTAL METHODS

The experiments were performed on cats and rabbits, both during aseptic operations, to prepare the animals for long term experiments, and conditons of short term experimentation. In all, we performed 33 experiments on 29 cats and 4 rabbits. In addition, we followed the course of oxygenation of blood in 3 control animals which had not been operated on, but were merely immobilized on the bench for the same length of time as were the experimental animals. We measured pulmonary ventilation in 11 short-term experiments before and 10-25 minutes after bilateral denervation of the carotid sinus regions.

Oxygen saturation of arterial blood was measured by the method described in Part I of this series [1]. The volume of expired air was measured by collecting it in a thin rubber bag for 3 minutes (through water valves with a resistance to inspiration and expiration of 5-7 mm of water), by replacement of water in a connecting graduated vessel. We calculated the volume of air respired per minute.

The experiments on cats were conducted under Evipan narcosis. The evipan was given by intramuscular injection (0.1 per kg body weight). Local anesthesia with 2% procaine was used in experiments with rabbits.

EXPERIMENTAL RESULTS

a) Denervation of Carotid Sinus Regions

Denervation of the carotid sinus regions was followed in all cases by a fall in the mean level of saturation of the blood with oxygen. The effect of unilateral division of a sinus nerve was relatively small. Bilateral

division of the carotid sinus nerves was followed by a marked fall in the degree of saturation of arterial blood with oxygen; it remained at the same low level during the whole of the period of observation. Administration of pure oxygen for 20-30 minutes after the operation raised saturation to 99-100%, but when it was replaced by air saturation, fell to the low level previously observed. Pulmonary ventilation diminished after denervation in all of 9 cats and 2 rabbits examined. Whereas in cats, saturation of arterial blood with oxygen fell from a mean value of 96.5% to 89-90% 5-10 minutes after denervation, in rabbits, the fall was from 95% to 86.5%. Pulmonary ventilation, which in cats amounted to 750 cc before denervation, fell after 20 minutes to 530 cc. Similar changes were found in rabbits.

We observed various transient fluctuations in oxygen saturation during preparation of the animals for operation and during the operation itself. These changes appeared in conjunction with various operative manipulations (skin incisions, exposure of nerves and vessels, pulling up of vessels preparatory to ligation, etc.), and also with violent movements of the animals. These were particularly evident in rabbits, which were not operated under general anesthesia.

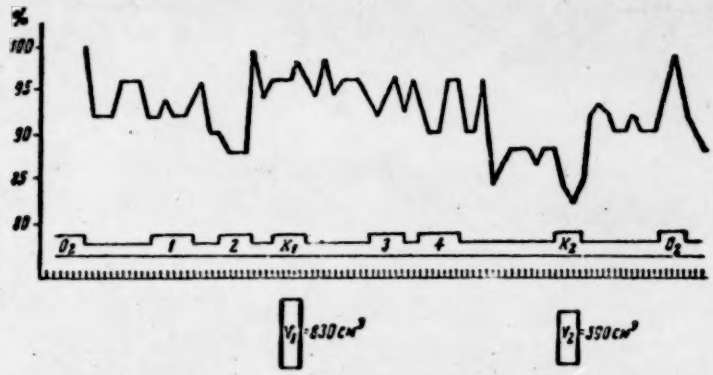

Fig. 1. Oxygen saturation of arterial blood, and pulmonary ventilation after denervation of the carotid sinuses (short-term experiment of May 25, 1956). Ordinates: percentage saturation; abscissae: time in minutes, and experimental conditions. Explanation of markings (from left to right): O_2 – setting of the instrument for 100% saturation when breathing pure oxygen; 1) skin incision and preparation of the neurovascular bundle; 2) preparation of the bifurcations of the left and right common carotid arteries; K_1 – measurement of pulmonary ventilation (breathing through inlet and outlet valves); 3) denervation of the right carotid sinus; 4) denervation of the left carotid sinus; K_2 – measurement of pulmonary ventilation; O_2 – breathing pure oxygen after denervation.

An example of the changes in oxygen saturation during a short term experiment with a cat is given in Fig. 1. The figure shows the fluctuations in oxygenation of the blood during the period of preparation for denervation, and at the moment of denervation (skin incision, preparation of the common, internal, and external carotid arteries, and division of the sinus nerves).

Changes in pulmonary ventilation are shown below the time axis. It is apparent that even a slight resistance to collection of expired air after denervation led to a considerable fall in oxygen saturation of the blood. The mean fall in oxygen saturation following denervation of this animal was from 96 to 90%, and in pulmonary ventilation from 830 to 590 cc. Still greater changes were found for rabbits.

It is thus evident that denervation of the carotid sinus regions uniformly leads to development of a hypoxemic state of the animals, and to a fall in pulmonary ventilation.

b) Denervation of the Spleen, and Division of the Femoral Nerves

Measurement of oxygen saturation of the blood during denervation of the spleen, performed for the purposes of a long term experiment, also revealed certain changes. These appeared both during division of the splenic nerves and during irritation of the peritoneum. The fluctuations in oxygen saturation were, however, only transient ones, and in general oxygen saturation of the blood had reverted to the preoperation levels with 15 minutes of division of the nerves.

Division of the femoral nerves had only an insignificant effect on oxygen saturation, which remained at a practically constant level.

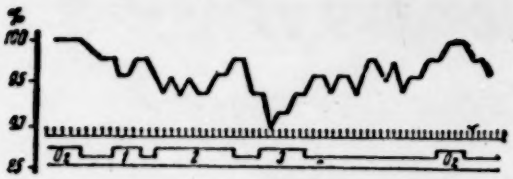

Fig. 2. Oxygen saturation of arterial blood following denervation of the spleen.
Ordinate – percentage saturation; abscissae – time in minutes, and experimental conditions. Explanation of markings (from left to right): O_2 – setting of instrument for 100% saturation when breathing pure oxygen; 1) skin incision; 2) opening of the abdominal cavity and exteriorization of the spleen; 3) division of the splenic nerves; O_2 – breathing of pure oxygen after denervation.

Figure 2 illustrates the changes in oxygen saturation of arterial blood during the operation of denervation of the spleen. A certain fall in saturation is evident at the moment of division of the splenic nerves and during insertion of stitches into the peritoneum. After the operation saturation rose to the initial level, amounting to 96-98%, and rose further to 100% during oxygen breathing. No appreciable changes in saturation followed division of the femoral nerves, except for a transient fall at the moment of division of the nerves (Fig. 3). Saturation of arterial blood is here practically the same as seen in the control experiments on the animals tied to the operating table, but not operated on. It follows that neither denervation of the spleen nor division of the femoral nerves cause any appreciable persistent changes in oxygen saturation of arterial blood.

Fig. 3. Oxygen saturation of arterial blood after division of the femoral nerves.
Ordinate – percentage saturation; abscissa – time. The fluctuations seen half-way through the experiment appeared at the moment of division of the nerves.

DISCUSSION OF RESULTS

Denervation of the carotid sinus regions caused a pronounced fall in pulmonary ventilation and led to a marked hypoxemic state of the experimental animals. Division of other nerves (splenic, femoral), and also infliction of other powerful stimuli, such as laparotomy, for example, caused disturbances of oxygen saturation of only very short duration.

It is known that respiration is one of the most labile functions of the organism, and is readily affected by different factors acting on the organism. It was to be expected that operations involving stimulation of sensory nerves or of the peritoneum would cause transient changes in respiration, and would affect the degree of saturation of arterial blood. Exclusion of such massive receptor fields as are the carotid sinuses, which participate constantly in the regulation of respiration, caused persistent respiratory disturbances, manifested in lowering of pulmonary ventilation. This effect was probably the cause of the lowering of oxygen saturation of arterial blood.

Our results point to a connection between oxygen saturation of blood and changes in pulmonary ventilation, and are evidence that the regulation of the degree of oxygen saturation of arterial blood is effected by means of reflex action on the respiratory function.

SUMMARY

Denervation of sinocarotid zones causes a stable decrease in the saturation of the arterial blood by oxygen and a decrease of the volume of lung ventilation in cats and rabbits. Only short decrease of saturation was noted during denervation of the spleen and removal of the femoral nerves. This was due to the section of the nerves and irritation of the peritoneum. An important role is played by the reflex component in regulation of the saturation of the arterial blood by oxygen.

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EFFECT ON RENAL FUNCTION OF HEMISECTION OF THE SPINAL CORD OF DOGS

COMMUNICATION II. EFFECTS ON RENAL FUNCTION OF REPEATED HEMISECTION OF THE SPINAL CORD IN THE CERVICAL REGION

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We showed in our previous paper [1] that hemisection of the spinal cord at the C-1 and C-5 levels causes disturbances of renal function. The severity of these disturbances varies according to the level of the spinal lesion.

The present research was undertaken with the object of elucidating the nervous mechanisms which enters into the process of compensation of the disturbances of renal function due to a single hemitransection of the cervical spinal cord, and also of obtaining confirmatory evidence of the dependence of the severity of the disturbances on the level at which hemisection of the cord was performed.

EXPERIMENTAL METHODS

A second hemisection was performed on 4 dogs, which had previously suffered the same operation, on the same side. Where the earlier lesion had been inflicted at the level of the 5th cervical segment, the second one was at the level of the 1st segment, and vice versa. Only 2 dogs were studied after the second operation, since one (Belka) died on the operating table, and experiments on another dog, Ryzhyi, had to be abandoned owing to development of multiple papillomatous growths around the ureteric orifices of the bladder. The second hemisection was performed at the level of the 1st cervical segment in Kashtanka, and of the 5th segment in Zolushka.

The activities of the right and left kidneys were compared in the same way as described in Part I [1], with the difference that the creatinine content of the urine was not determined.

EXPERIMENTAL RESULTS

The changes in renal function observed after repeated hemisection of the right side of the spinal cord closely resembled those seen after the first operation, and their nature and severity depended on the level of the operation. The output of the right kidney of Zolushka was much less than of the left one, on the second day after repeated hemisection at the level of the 5th cervical segment, while the concentrations of urea and chloride were higher. From the 5th day on, we observed a steep fall in the concentration and amount of chloride cleared through the right kidney; output remained low, and urea concentration high. On the 2nd day after the operation neither the left nor the right kidney reacted to water loading by increased diuresis, although the duration of the experiment was 3 hours. On the 5th day after hemisection both kidneys gave only a small diuretic response to water loading, although 600 ml of water were introduced into the stomach, instead of the usual 400 ml. Only by the 8th day did both kidneys respond adequately to functional loading.

Beginning with the 2nd day after hemisection, Kashtanka showed a certain rise in diuresis on the operated side (average increase over the whole period of observation of 10-12%, but 1.5-1.8 times greater for individual 30-minute portions of urine), with correspondingly lower concentration of urea and chloride in the urine of this kidney, as compared with that of the contralateral one.

The time required for restoration of function was shorter after the second hemisection than after the first one. The disturbance of renal function following the second hemisection, above or below the level of the first one, lasted for less than two weeks, i.e., restoration of function was achieved nearly 5 times faster than after the first operation. Full restoration of the disturbed somatic functions following the second operation took place within 25-30 days, i.e., 1.3-1.4 times faster than after the first operation. Protein was absent from the urine of both kidneys after the second hemisection.

DISCUSSION OF RESULTS

We have shown that hemisection of the spinal cord causes considerable disturbances of renal activity, which is an indication of the existence of nervous regulation of kidney function.

Hemisection of the spinal cord at the level of the 5th cervical segment caused more severe disturbances than at the level of the 1st segment. Evidently the disturbances of function of the spinal sympathetic centers resulting from surgical trauma are greater when the wound is inflicted at a lower than at a higher level.

Our findings indicate that disturbances in renal function following hemisection of the spinal cord may be conpensated, similarly to disturbances in somatic functions. Restoration of somatic functions is, however, achieved in half the time that is required for restoration of normal renal function. This difference is probably related to the specific functional characteristics of the spinal somatic and vegetative reflex reactions, and in particular to the high inertia of the latter, and to their relatively smaller dependence on the higher levels of the central nervous system, which have been shown by E. A. Asratian [2] and his associates to play an important part in the restoration of disturbed functions of an injured organism.

What is the mechanism of restoration of disturbed renal function after hemitransection of the spinal cord?

It is generally believed that the process of restoration of functions of the higher animals following infliction of lesions of the spinal cord is not a result of regeneration of divided intracentral pathways. The relative rates of restoration of disturbed functions, and the results of macro- and microscopic examination of spinal cord specimens from Belka and Ryzhyi confirm this belief. It may therefore be assumed that participation of the higher levels of the central nervous system in the process of restoration of disturbed renal functions (if it does actually occur) is achieved through the uninjured conducting pathways of the contralateral side of the spinal cord, in the same way as for restoration of disturbed somatic functions.

Restoration of normal functioning of the kidneys, similarly to restoration of somatic functions, proceeds gradually, and shows none of the signs of automatic reconstruction. The gradual nature of the process of restoration of renal function may indirectly indicate that a training mechanism is concerned, or that various levels of the brain exert an action on the kidneys, or that both of these mechanisms are involved.

The shorter time required for restoration of renal function after repeated spinal transection (about one-fifth of the time needed after the first operation) also supports the view that restoration of renal function depends not only on changes proceeding within the spinal cord itself, but also on the participation in this process of various formations of the brain.

SUMMARY

Experiments were performed on 2 dogs with chronic fistulae of the ureters. Hemisection of the spinal cord was previously performed in these dogs on the level of the segments and later the disturbed function of the kidney was re-established. After repeated hemisection, as well as after the first one, the degree of disturbance depended on the level of the section. Re-establishment of the kidney function following the second operation took place 5 times faster than after the first operation.

TABLE

Diuresis and Urea and Chloride Contents of 30-Minute Portions of Urine Before and After Repeating a Right-sided Hemitransection of the Spinal Cord at the Level of the 5th Cervical Segment of the Dog Zolushka, with Water Loading

Diuresis, in ml			Urea, %			Chloride, %		
left kidney	right kidney	ratio	left kidney	right kidney	ratio	left kidney	right kidney	ratio
Before the second operation (98th day after the first, level of C_1)								
7.5	8.5	100:113	3.26	2.886	100:88	0.435	0.395	100:90
Load 400 ml water								
11.5	14.0	100:121	1.843	1.606	100:87	0.242	0.201	100:84
37.0	46.0	100:124						
43.6	51.0	100:116						
42.0	46.0	100:109	0.437	0.437	100:100	0.035	0.032	100:91
16.5	19.5	100:128						
7.5	8.2	100:109	1.417	1.417	100:100	0.163	0.146	100:90
2nd day after operation								
4.5	3.2	100:71	2.691	6.247	100:232	0.189	0.292	100:154
Load 400 ml water								
4.9	3.4	100:69	2.499	4.854	100:194	0.213	0.347	100:163
5.0	3.5	100:70						
4.3	3.0	100:69	2.835	5.478	100:193	0.137	0.265	100:191
5.0	3.5	100:79						
4.5	3.2	100:71	2.806	4.998	100:178	0.078	0.154	100:187
5th day after operation								
5.5	3.6	100:65	1.422	2.631	100:185	0.163	0.128	100:78
600 ml water load								
5.7	5.4	100:94	1.47	2.75	100:180	0.146	0.07	100:48
9.0	6.5	100:72						
14.0	9.0	100:64	0.853	1.588	100:186	0.029	0.023	100:79
13.0	8.7	100:66						
11.0	7.4	100:67						
9.0	6.0	100:66						
7.4	5.3	100:82	1.28	2.465	100:197	0.216	0.137	100:63
13th day after operation								
5.0	7.0	100:140	2.628	2.26	100:86	0.263	0.219	100:83
Load 400 ml water								
5.5	6.7	100:121	2.699	2.482	100:91	0.21	0.192	100:91
11.0	15.7	100:142						
40.0	51.5	100:128						
51.5	57.7	100:112	0.355	0.307	100:86	0.04	0.04	100:100
27.5	32.5	100:117						
13.5	18.0	100:133						
7.0	9.5	100:135	1.515	1.478	100:97	0.245	0.213	100:87

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** In Russian.

ABSORPTION OF RADIOACTIVE IRON AND ITS ASSIMILATION BY ERYTHROCYTES IN ANEMIA CAUSED BY PARTIAL DENERVATION OF THE STOMACH

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Experiments involving the use of radioactive iron have shown that considerable absorption of iron takes place from the digestive tract of dogs suffering from various forms of anemia, and that this process is virtually absent in control animals [11, 12]. The radioactivity of the blood plasma reaches a maximum 1-2 hours after administration of iron, and is totally absent a few hours later. It has been established that the intensity of iron metabolism is determined in the first place by the functional requirements of the organism [1, 6, 9, 10].

We have applied radioactive isotopes to the study of various forms of experimental anemia caused by denervation of certain viscera, and described by V. N. Chernigovskii and his associates [8].

The present paper gives data relating to the absorption of the radioactive iron isotopes Fe^{59} (as $Fe^{59}Cl_3$) from the alimentary tract, and to its utilization for the synthesis of hemoglobin in dogs rendered anemic by partial denervation of the stomach. As a control of the absorptive power of the alimentary tract we also applied the previously obtained data [2] for absorption of radioactive phosphorus (given as $Na_2HP^{32}O_4$).

EXPERIMENTAL METHODS

We examined the absorption of iron and the rate of its incorporation into hemoglobin:

1. In healthy dogs, before operation (dogs Beliak and Malysh).

2. In dogs during the stage of hypochromic anemia supervening after operation for a Pavlov pouch (control, Malysh) or a Klemensiewicz-Heidenhain pouch (partial denervation; the dog Veselyi). The dog Veselyi was further studied during the stage of development of hyperchromic macrocytic anemia.

3. In dogs in which both vagus nerves have been divided below the diaphragm during the stage of hyperchromic macrocytic anemia, and after normalization of the blood picture (Bars, Tarzan, Fokus), and in a control dog in which the esophagus had been exposed below the diaphragm, without injury to the nerves (Beliak). In addition, we examined absorption of P^{32} in the dogs Bars and Tarzan. As controls for these experiments we used the healthy dogs Zhuchka and Rozka.

The radioactive isotopes were administered orally, in 250 ml of milk, at the dosage levels: iron – 1000 cpm phosphorus – 500 cpm per 1 g body weight.

Blood samples were taken 15, 30, 60, 90, 120, 180, 240, and 300 minutes after administration of isotopes, on the first day of administration, and thereafter once daily for the 3-5 days following, and then once in 4-8 days. Each animal was observed over a period of 4-8 months. Radioactivity was measured by means of a Type B counter. The mean error of the measurements did not exceed 3-4%.

We calculated the percentage of the given dose per 1 g body weight found in 1 ml of plasma, and the rate of incorporation of Fe^{59} into hemoglobin from the percentage of the given dose per 1 g body weight found in the erythrocytes of 1 ml of blood (taking into account the hematocrit readings). Serum radioactivity after administration of P^{32} was measured by means of a B-2 counter, and total phosphorus was determined colorimetrically. The specific activity of total serum phosphorus was then calculated. Blood morphology and biochemistry were studied parallel with these measurements [2].

EXPERIMENTAL RESULTS

1. In healthy dogs radioactive iron was found in the plasma 15-30 minutes after its introduction, and reached peak concentration within 120-240 minutes, diminishing gradually thereafter. The radioactivity of the plasma was negligible after 48-72 hours (Fig. 1, 1, 3).

Radioactivity appeared in the erythrocytes on the 4-5th day after administration of iron, and was maximum on the 8-9th day, remaining at this level for a long time, and then falling gradually. After 4 months the Fe^{59} content of the erythrocytes did not exceed 1% (see Fig. 1, 1a, 3a).

2. Post-operation hypochromic anemia developed in the dog Malysh after a Pavlov pouch operation (erythrocytes 3,560,000, hemoglobin 47%); the condition lasted for 1½ months. An analogous form of anemia supervened in the dog Veselyi after operation for a Klemensiewicz-Heidenhain pouch (erythrocytes 2,880,000; hemoglobin 28%). The erythrocyte count and the hemoglobin content rose slightly after 1½ months, but, in contrast to Malysh, reestablishment of the initial levels was not achieved. The erythrocyte count was 1-1.2 million below normal, and the hemoglobin content remained low by 14-18%. After 3½ months the anemia was of the hyperchromic macrocytic type, and persisted throughout the period of observation (8 months).

Radioactive iron was administered to both dogs during the postoperational phase of hypochromic anemia. Radioactivity measurements revealed pronounced intensification of absorption. On the first day of administration the plasma Fe^{59} level exceeded the preoperational one by 4-4½ times, and remained at a high level up to 5-6 days (see Fig. 1, 4, 5). The radioactivity of the erythrocytes was also 7-12 times higher than before the operation, reaching a maximum on the 6-7th day. It remained at this level for a very long time (over 80 days; see Fig. 1, 4a, 5a).

A second dose of radioactive iron was administered to the dog Veselyi during the phase of hyperchromic macrocytic anemia. As is evident from curve 6 (Fig. 1), absorption of iron was much lower, although still higher than in normal dogs. A similar effect was observed with respect to radioactivity of erythrocytes (see Fig. 1, 6a). It follows that incorporation of iron into hemoglobin proceeded more intensively than in healthy dogs, but less so than during the postoperational phase of hypochromic anemia.

3. After subdiaphragmatic division of the vagus nerves of the dogs Bars, Tarzan, and Fokus hyperchromic macrocytic anemia developed, lasting for 3-4 months. The erythrocyte count fell by 1.5-2 million, and the hemoglobin content by 15-22%.

Radioactive iron was administered to these dogs, at the height of the anemic phase. The radioactivity of the plasma was found to be 3-4 times lower than in normal dogs; neither on the following day nor thereafter did we find even traces of activity (Fig. 2, B, 1-3). The erythrocytes displayed activity on the 3rd and 5th days after administration of iron, and, as with the preceding dogs, the activity rose to a maximum on the 6-7th day, although its level was considerably lower (see Fig. 2, B, 4a, 2a, 3a). It may be concluded that, despite the development of anemia, division of the vagus nerves is associated with a sharp fall in absorption of iron and in its assimilation by erythrocytes. A similarly pronounced retardation of absorption of P^{32} from the alimentary tract was observed for these same dogs. This is clearly shown by comparison with the data for control dogs (see Fig. 2, A, 1, 2, and 3, 4). After a second dose of radioactive iron we again found inconsiderable absorption from the alimentary tract (see Fig. 2, B, 4, 5).

Measurements of the radioactivity of feces collected during 3 days (a representative sample was taken, and an aliquot of its suspension in water was taken for radioactivity measurement) showed that 88% of the administered iron was excreted.*

* The actual figure is probably still higher, as the radioactivity of the contents of the intestines is not included in it.

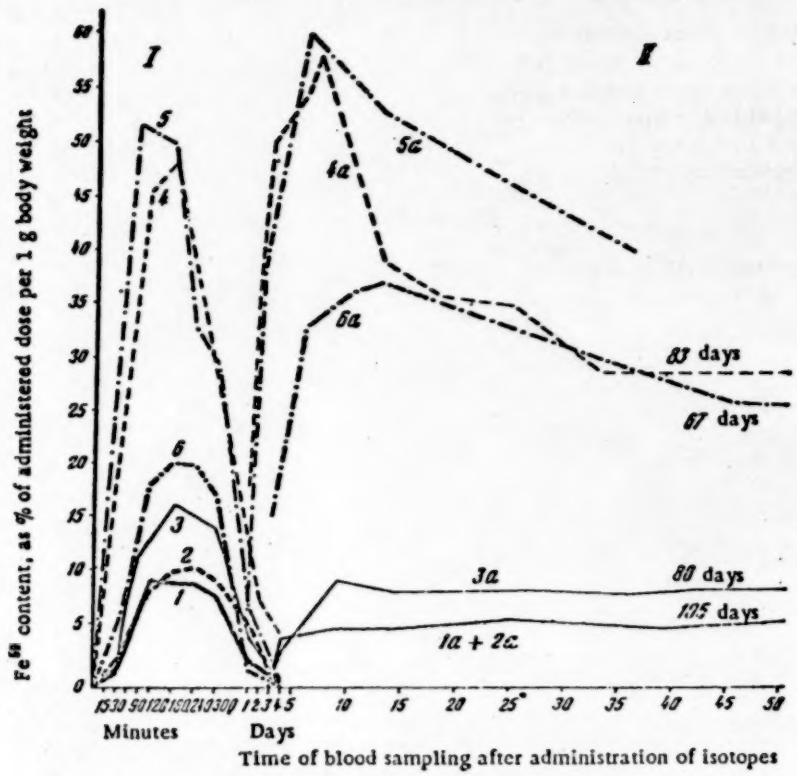

Fig. 1. Rate of absorption and assimilation of Fe^{59} in dogs, before and after partial denervation of the stomach.
I) Velocity of absorption of Fe^{59} from the alimentary tract; 1) Beliak, before operation; 2) Beliak, after laparotomy; 3) Malysh, before operation; 4) Malysh, in the postoperational phase of hypochromic anemia (Pavlov pouch); 5) Veselyi, in the postoperational phase of hypochromic anemia (Klemensiewicz-Heidenhain pouch); 6) Veselyi, in the hyperchromic anemia phase. II) Rate of incorporation of Fe^{59} into hemoglobin: 1a, 2a) Beliak, before and after operation; 3a) Malysh, before operation; 4a) Malysh, in the postoperational phase of hypochromic anemia; 5a) Veselyi, in the postoperational phase of hypochromic anemia; 6a) Veselyi, in the hyperchromic anemia phase.

The dogs were killed, and the iron content of the liver and spleen (homogenates) was determined. The liver was found to contain about 2% of the iron administered, and the spleen 0.7%. Thus vagotomy caused a pronounced lowering of absorption of iron.

Our findings show that absorption of Fe^{59} from the alimentary tract is considerably enhanced during the postoperational phase of hypochromic anemia, as is also its assimilation for synthesis of hemoglobin. Absorption falls somewhat with transition from hypochromic to hyperchromic anemia, but still remains higher than normal. The high levels of radioactivity of erythrocytes persists for a long time in these forms of anemia.

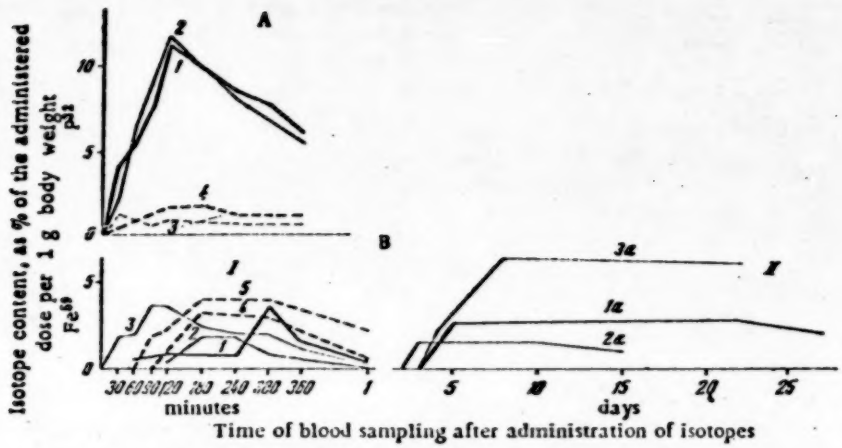

Fig. 2. Rates of absorption and assimilation of radioactive isotopes by dogs after subdiaphragmatic division of the vagus nerves (hyperchromic anemia). A) Rate of absorption of P^{32} from the alimentary tract; 1) and 2) control dogs Rozka and Zhuchka; 3) and 4) operated dogs Bars and Tarzan (vagotomy). B. I) Rate of absorption from the alimentary tract during the phase of hyperchromic macrocytic anemia; 1) and 4) Bars; 2) Tarzan; 3) and 5) Fokus; B. II) Rate of incorporation of Fe^{59} into hemoglobin during the phase of hyperchromic macrocytic anemia; 1a) Bars; 2a) Tarzan; 3a) Fokus.

The onset of hypochromic anemia after stomach pouch operations can scarcely be ascribed solely to loss of blood incidental to them; it is therefore hardly possible to agree with P. Hahn's explanation of the raised absorption of iron [11, 12]. The shape of the curves expressing incorporation of iron into the erythrocytes indicates that this is associated with a fall in the mean survival time of the erythrocytes. Iron is more rapidly utilized for hemoglobin synthesis than normally, but it is also more rapidly liberated from the erythrocytes. This is evidence of enhanced hemolysis. Further evidence of this is afforded by a number of other observations, such as the presence of microspherocytosis, changed osmotic resistance of the erythrocytes, the low color index, etc.

In dogs operated for a Klemensiewicz-Heidenhain pouch, which is considered to involve partial denervation, full recovery from the postoperational anemia does not take place, and it gradually undergoes transition to the hyperchromic macrocytic form. The fragility of the erythrocytes becomes smaller, the color index rises, and maturation of erythrocytes is considerably retarded (bone marrow smears).

Absorption of iron falls during the hyperchromic anemia phase, and approaches the values found before operation. The rate of incorporation of iron into hemoglobin also falls somewhat, although it still remains higher than before operation. The survival time is considerably prolonged, approaching the normal value. There can, therefore, be no question of enhanced hemolysis during this phase. Nor can we consider that there is any lack of iron.

We consider that the gradual onset of hyperchromic anemia is due to disruption of nervous connections between part of the stomach and the central nervous system. This interpretation is based on V. N. Chernigovski's views [8] on the importance of continuous tonic impulses proceeding from the organs to the central nervous system, for the maintenance of the constant composition of the blood.

Bilateral vagotomy is followed by a considerable fall in absorption from the alimentary tract, and in assimilation of iron by erythrocytes. The same effects are observed with respect to phosphorus. Most of the

iron is excreted in the feces, and only a small part of it enters the circulation. The anemia which develops in these circumstances is of the hyperchromic macrocytic form, within the first few days after operation. Pronounced retardation of maturation of erythrocytes is observed. We may, in the given case, postulate a certain lack of iron.

Vagotomy not only interrupts the afferent connections of the stomach, but also leads to considerable disturbances in alimentary tract functions, and to development of structural changes; formation of the intrinsic antianemic factor by the gastric mucosa is interfered with [4, 5, 7]. We cannot exclude the possibility that absorption from the alimentary tract, not only of iron and phosphorus, but also of other products, including the antianemic factor, may be impeded, and this may be one of the causes of the anemia observed.

SUMMARY

Long term experiments were performed on dogs with experimental anemia induced by denervation of the stomach (by Klemensiewicz-Heidenhain partial denervation or by section of the vagus nerves). Investigation was carried out on the absorption of radioactive iron ($Fe^{59}Cl_3$) in the digestive tract and its intake by the erythrocytic hemoglobin.

It was established that absorption and intake of iron is considerably increased during postoperative hypochromic anemia in experiments with the stomach pouch. When hypochromic anemia is changed into hyperchromic the intake of iron by erythrocytes is decreased.

Development of hyperchromic anemia is noted after section of the vagus nerves. At the same time absorption and intake of iron is greatly decreased.

Absorption of radioactive phosphorus (P^{32}) is also diminished.

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** Original Russian pagination. See C. B. Translation.

EFFECT ON CAPILLARY PERMEABILITY OF SINGLE AND REPEATED OVERHEATING OF AN ORGANISM

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Numerous papers have been published [1, 4, and others], dealing with the effects of overheating of an organism on the permeability of various of its membranes and structures, including the capillary walls; data have also been published on the effect of hyperthermia on the permeability of the hemato-encephalic barrier [2, 7, and others]. Insufficient attention has, however, been paid to the significance of the permeability factor as an adaptive function to the action of high temperatures, and virtually no data are available relating to the adaptation of permeability functions to repeated exposure to high temperature. The present paper deals with a study of this problem.

EXPERIMENTAL METHODS

We applied the method of luminescence analysis, as elaborated by V. A. Iusin [5], to the study of capillary permeability. As the indicator we used a 1% solution of Akrikhin* hydrochloride (Mepacrine), which was injected subcutaneously at a rate of 50 mg per kg body weight. Permeability was derived from the rate of resorption of Akrikhin from the site of injection, and from its concentration in various organs after the lapse of various periods of time. Akrikhin concentration was determined in the brain, lungs, liver, kidneys, spleen, heart muscle, gastric and intestinal walls, and blood. The experimental animals were white rats, aged 8-12 months, mostly females, weighing 150-200 g. The rats were heated in special thermal chambers (dimensions 0.8 × 0.8 × 1.5 m), at 45°. We used 80 rats, on which we performed 801 experiments, in addition to the appropriate control experiments.

EXPERIMENTAL RESULTS

Our preliminary experiments [6] showed that a single exposure to heat, involving a rise in body temperature to 42-43°, was associated with a considerable rise in capillary permeability in the brain and other organs. No such rise in capillary permeability was seen when narcotized rats (sodium amytal) were similarly exposed, notwithstanding a rapid rise in rectal temperature to 42-43°.

It may be supposed that the inhibitory effect of the drug on the central nervous system of hyperthermic animals is exerted in the given case on the important adaptive mechanism of increase in capillary permeability. According to N. N. Konstantinova's findings [3], hyperthermia develops more rapidly in rats under barbiturate narcosis, and runs a more severe course; the survival rate of the drugged rats is much lower than is that of rats which are awake at the time of exposure.

Our further experiments were directed towards finding out how soon after exposure to heat did the permeability of the capillaries rise in various organs, and particularly in the lungs, since it is chiefly through these that thermoregulation is effected in rats, and whether the heightened capillary permeability remained at the same level throughout the whole of the period of exposure to heat. The rats were placed in the thermal chamber at 45°, and the permeability of the capillaries to Akrikhin was examined separately over two 15-minute

*Russian trade name for Quinacrine hydrochloride, Editor.

periods. During the first period the indicator was injected before exposure to heat, with a normal body temperature, and permeability was assessed after 15 minutes exposure to heat.

As is shown in the Table, the Akrikhin content of the organs, particularly of the lungs, was relatively high, despite the short time elapsing after its injection. The rectal temperature of the rats had risen by 1-1.5° during the 15 minutes in the chamber. It remained uncertain whether capillary permeability had risen in response to the rise in body temperature, or whether it had risen reflexly before the rise in temperature had taken place. Our experiments showed that under our experimental conditions rise in body temperature began at the 11-13th minute of heating. In our further experiments we examined capillary permeability 7-8 minutes after exposure to heat, i.e., at normal body temperature. In these experiments our attention was directed chiefly to the pulmonary capillaries. By this time, the ears of the rats were hyperemic, indicating the beginning of the adaptive thermoregulatory reaction to high environmental temperature.

It follows from our experiments (see Table) that the permeability of the lung capillaries also rises under these conditions, although that of other viscera remains unchanged. These experiments appear to provide evidence that the changes in permeability of capillaries, particularly of those of the lungs, are among the first adaptive mechanisms to come into action in response to high environmental temperatures.

In the second series of experiments Akrikhin was injected when the rats were already hyperthermic, 15 minutes after being placed in the chamber. They were kept in the chamber for a further 15 minutes, and were then killed.

The results of luminescence analysis (see Table) also showed a raised Akrikhin content of the organs. Comparison with the results of the first series of experiments shows that when Akrikhin was given to rats with a raised body temperature its concentration rose much more within the organs in question. This applied not only to the lungs, but also to other organs; Akrikhin was found in tissues from which it had been absent in the animals of the first series. This suggests that the rise in capillary permeability observed during the first phase of heating, in particular in the lungs, is of the nature of a protective-compensatory mechanism, facilitating adaptation of the organism. However, with further prolongation of the action of heat, as an extraordinary stimulant, a breakdown, or an overstraining of these protective-adaptive mechanisms takes place, and the organism begins to react in a pathological manner, in the given case in the form of an excessive ("uneconomical") heightening of permeability of the capillaries of the lungs and of many other organs.

A "permeability breakdown" of this sort may facilitate leakage of plasma proteins into the tissues, with plugging of capillaries, enhanced viscosity of the blood, and weakening of cardiac activity, as well as passage of blood cells through the capillary walls into the substance of the brain and other organs.

It is of interest that when Akrikhin is injected into an already hyperthermic animal its concentration in various organs 15 minutes later exceeds that found after 27-30 minutes of exposure to heat when the injection was made into animals having a normal body temperature. This effect may be explained as being due to the increased rate of resorption of Akrikhin when it is injected into an animal with modified reactivity, due to the preceding period of "stimulation," as a result, Akrikhin readily enters the blood stream, out of which it rapidly diffuses into the tissues.

We performed 2 series of experiments on 30 rats, with the object of investigating permeability adaptation to repeated exposures to high environmental temperatures. In the first series, the rats were placed daily for 20 minute periods in a chamber at 45°. In the second series the temperature of the chamber was raised gradually, at a rate of 2° daily, from an initial temperature of 20° to a final one of 45°. Akrikhin was injected before the last exposure at 45°, and the rats were killed 27 minutes later.

The results of luminescence analysis (see Table) showed that, although the capillary permeability of these rats was heightened, it was much smaller than after a single exposure. This effect may to a certain extent be taken as evidence that gradual adaptation of the organism takes place with repeated exposure to high temperature. Such adapted animals are able to maintain normal body temperature for a longer time than unadapted ones, making them better able to withstand high environmental temperatures.

It should be noted that the results of the two series of experiments were identical. Hence it follows that whether the animals are habituated by exposure to high environmental temperature from the first day of conditioning, or whether by exposure to gradually rising temperatures, the same final conditioning effect is achieved, manifested by adaptation of permeability processes.

TABLE

Akrikhin Content of Various Organs of White Rats Subjected to Overheating in a Thermal Chamber at 45°

Experimental conditions	Time after injection Akrikhin (min)	Akrikhin content (mg-%)									Mean rise in body temp.
		spleen	kidneys	liver	lungs	brain	intestine	stomach	heart	blood	
Control (animals not exposed to heat)	27	0.016	0.102	0.112	0.198	0	0	0	0.185	0.06	—
Single heat exposure	27	0.19	0.31	0 25	1.9	0.05	0.14	0	0.36	0.0625	4°
Single heat exposure in narcotic sleep	27	0.025	0.08	0.078	0.34	0	0	0	0.137	0.044	4.6°
Repeated heat exposures	27	0.078	0.166	0.13	0.88	0	0.018	0	0.16	0.078	3.1°
Repeated exposure, raising chamber temp 2°	27	0.075	0.145	0.145	0.675	0	0	0	0.173	0.06	3.2°
Single heat exposure. Akrikhin injected into rats with normal body temp.	15	0.078	0.14	0.2	1.4	0	0	0	0.218	0.05	1.5°
Single heat exposure. Akrikhin injected into rats with raised body temp.	15	0.12	0.25	0.297	3.31	0.02	0.094	0.078	0.45	0.06	4.2°
Control experiments	15	0	0	0	0.0625	0	0	0	0	±	—
Single heat exposure	7	0	0	0	0.3	0	0	0	0	±	0°
Control experiments	7	0	0	0	0	0	0	0	0	±	—

Thus the effect of high temperature on white rats is to cause a considerable heightening of capillary permeability, in which reflex processes play an important part. Heightening of permeability of lung capillaries takes place very early, together with other compensatory mechanisms (such as hyperemia of the ears, for example), which may be an indication of the adaptive role of this factor.

Overheating of the organism causes a marked rise in permeability of the capillaries of the brain and other viscera to Akrikhin.

The rise in capillary permeability following exposure to heat takes place, particularly in the lungs, before there is any rise in body temperature; this may be evidence of the part played by pulmonary capillary permeability in the process of adaptation of animals to high environmental temperatures.

The rise in capillary permeability observed following multiple exposures to high environmental temperature is smaller than after a single exposure.

SUMMARY

Experiments were performed on white rats. It was demonstrated that overheating is connected with significant increase of permeability of blood capillaries in internal organs and in the brain to Akrikhin. Reflex processes play an important role here and the change of permeability depends on the functional condition of the organism.

Increase of permeability of lung capillaries, as well as other compensatory mechanisms takes place prior to increase of the body temperature. This, evidently, points to the adaptive role of this factor. After repeated overheating the increase of the vascular permeability is less than when it is done only once. This shows that the factor of permeability adapted itself to overheating.

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EFFECT OF SOME BACTERIAL INTOXICATIONS ON THE CHOLINESTERASE ACTIVITY OF RATS' BRAIN

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Changes in the reactivity of the central nervous system are an important feature of infectious diseases. It may be supposed that such changes in reactivity are a consequence of the action of bacterial toxins or enzyme systems of nerve cells. The elucidation of the mechanism whereby the functional state of the nervous system is affected in infectious diseases may in some cases afford the possibility of modifying enzymatic processes of nerve cells.

Much attention has been paid of recent years to cholinergic reactions, in the study of the pathology of infections. The importance of acetylcholine in modifying the reactivity of the nervous system has been established by a number of authors. To this may be ascribed the interest taken by research workers in changes in cholinesterase activity in infectious diseases.

We have studied cholinesterase activity of the brain of animals suffering from pneumococcal, dysentery, and tetanus intoxications.

We have been unable to trace any literature references to the effects of pneumonia on cholinesterase activity.

A very slight activation of cholinesterase has been reported to result from the direct action of Flexner type dysentery antigen [6]. A fall in cholinesterase activity of the blood serum [11] and of extracts of the brain and spinal cord [10] has been found in experimental dysenteric intoxication of animals. A similar effect has been noted for the serum of dysentery patients [6].

Tetanus toxin exerts an anticholinesterase action in vitro [8, 12, 13, 15]. M. B. Lebedeva and I. L. Chertkov [8] found no change in the cholinesterase activity of extracts of the brain and spinal cord in animals suffering from tetanus intoxication. In similar experiments, N. K. Fruentov [12] found a slight lowering of cholinesterase activity.

As appears from the literature references cited, the effects of the given intoxications on cholinesterase activity were studied using extracts of the whole brain. Bearing in mind the differences in physiological activity of different parts of the brain, we thought it would be more instructive to examine separately the cholinesterase activity of the grey matter of the cerebral cortex, of the part of the brain corresponding to the thalamo-hypothalamic region, and of the cerebellum.

EXPERIMENTAL METHODS

The experimental animals were male and female rats, weighing 100-200 g. Practically healthy rats were taken as controls for each series of sick animals. Pneumococcal intoxication was achieved by giving intradermal inoculations of titrated 18-hour cultures of Type I pneumococcus, which caused death within 2-3 days.

Cholinesterase activity was determined in seriously ill rats. Dysenteric intoxication was effected by intraperitoneal injection of a lethal dose of Flexner type dysentery complete antigen. The animals became seriously ill within 3-4 hours. Tetanus intoxication was achieved by subcutaneous injection into the left hind leg of tetanus toxin, at a dosage of 0.1 mg in 0.1 ml of physiological saline. This was usually followed by localized tetanus, on the 2nd and 3rd day, and the animals died on the 4-5th day, with symptoms of generalized tetanus. Cholinesterase activity was determined in material taken from rats killed shortly before they were expected to die.

The rats were decapitated, and the brain was removed as quickly as possible, cooled, and the requisite samples were removed, weighed, and ground up with cooled buffer solution (1 ml per 50 mg of tissue). Cholinesterase activity was determined manometrically, in a Warburg apparatus, according to D. Nachmanson and M. Rothenberg [14]. The activity was expressed as μl CO_2 evolved per mg of tissue during the first 30 minutes.

EXPERIMENTAL RESULTS

The results are presented in the Table. A tendency towards lowering of cholinesterase activity of the grey matter of the cerebral cortex was evident in pneumococcal intoxication. This decrease was not, however, statistically significant. No change was found in the cholinesterase activity of the thalamo-hypothalamic region.

The cholinesterase activity of the grey matter was raised by 37% in dysenteric intoxication; that of the thalamo-hypothalamic region and of the cerebellum showed no change.

Tetanus toxin raised the cholinesterase activity of cortical grey matter by 40%, and of the thalamo-hypothalamic region by 27%. No change was found in the cerebellum. The rise in cholinesterase activity was in all these cases statistically significant.

TABLE

Cholinesterase Activity of Different Parts of the Brain of Healthy and Sick Rats

Part of brain examined	M – μl CO_2 evolved in 30 min. per mg tissue (mean values; range given in parentheses)	n – number of experiment	m – mean error	Significance of deviations from normal	Percentage of deviations from normal
	Normal				
Cortex	2.7(1.6—3.8)	13	±0.1986	—	—
Thalamo-hypothalamic region	4.7(4.1—5.2)	9	±0.1532	—	—
Cerebellum	2.0(1.2—2.6)	8	—	—	—
	Pneumonia				
Cortex	2.2(1.4—3.1)	7	±0.2560	Not significant	—18
Thalamo-hypothalamic region	4.9(3.5—6.6)	7	—	—	—
	Dysentery				
Cortex	3.7(2.5—4.6)	11	±0.2322	Significant	+37
Thalamo-hypothalamic region	5.1(4.6—5.5)	9	—	—	—
Cerebellum	2.0(1.6—2.3)	7	—	—	—
	Tetanus				
Cortex	3.8(3.2—4.2)	8	±0.1180	Significant	+40
Thalamo-hypothalamic region	5.9(5.2—6.6)	8	±0.2070	Significant	+27
Cerebellum	2.2(1.9—2.7)	7	—	—	—

Our results differ somewhat from those published in the literature. This may be because the changes in cholinesterase activity found by us in particular parts of the brain might not be perceptible when the activity of the whole brain is studied. According to M. B. Aristovskii [2] the enhanced nerve conductivity found in tetanus is a consequence of interference with acetylcholine breakdown, and of its accumulation in the nervous system, owing to the inhibitory effect of tetanus toxin on cholinesterase.

D. E. Al'pern et al., [1] believe that increase in cholinesterase activity of the blood and tissues proceeds, within certain limits, parallel with increase in their acetylcholine content, and may thus be regarded as a compensatory function of the organism. In accordance with this view, G. E. Platonov [9] related the raised cholinesterase activity of the hypothalamic region, found by him in tuberculous intoxications to accumulation of acetylcholine-like substances. Thus our findings also support the view that accumulation of acetylcholine occurs in the grey matter of the cerebral cortex in dysenteric and tetanus intoxications, and in the thalamo-hypothalamic regions in tetanus intoxication; this may explain the raised reactivity of these parts of the brain in these conditions.

The conclusion may be drawn from our results that cholinesterase activity rises in the grey matter of the cerebral cortex and in the thalamo-hypothalamic region, in tetanus intoxication of rats; it does not change in the cerebellum.

Cholinesterase activity of rats suffering from pneumococcal intoxication remains unchanged in the cerebral cortex and in the thalamo-hypothalamic region.

SUMMARY

It was experimentally demonstrated that cholinesterase activity increases in the gray matter of the cerebral hemispheres in tetanus and dysentery intoxications. However, there is no change in its activity in pneumococcal intoxication. The activity of the enzyme does not change in the cerebellum in tetanus and dysentery intoxications. The increase in activity of cholinesterase in all the above cases is proved statistically.

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BIOCHEMISTRY AND BIOPHYSICS

THE MECHANISM OF "PARADOXICAL" INCREASE OF BLOOD DIAMINOXIDASE (HISTAMINASE) ACTIVITY UNDER THE INFLUENCE OF DIMEDROL AND THIAMINE

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Our experiments [2] on the effect of dimedrol on the activity of blood diaminoxidase (histaminase) showed that dimedrol did not always alter the activity of this enzyme in the same direction; it inhibited diaminoxidase activity but, in a number of cases, enhancement of such activity was noted.

We became interested in the question as to whether such difference in the character of the dimedrol effect depended to any extent on the species peculiarities of the experimental animal.

EXPERIMENTAL METHODS

The effect of dimedrol in concentration of 10^{-4} on the rate of histamine destruction was studied on blood from cows and pigs. Histamine was added to the blood to give concentrations of 10, 30 or 100 γ/ml. Incubation was carried out at 38° with constant shaking over a period of 4 hours. The amount of histamine remaining in the reaction mixture was determined by the depressor effect on cats anesthetized with urethane. Control experiments were set up simultaneously in which no dimedrol was added to the blood. The data obtained in the experiments was used to calculate the velocity constants for histamine destruction in order to characterize the activity of the enzyme. The reaction was considered to be monomolecular and the following formula was used for calculating the velocity constant for the reaction:

$$K = \frac{2.303}{t} \log \frac{a}{a-x},$$

where K – velocity constant for the reaction; t – time (in minutes) from the beginning of the reaction; a – initial amount of substrate (in γ); x – amount of substrate (in γ) destroyed.

EXPERIMENTAL RESULTS

The results of these experiments are presented in Table 1.

Table 1 shows that dimedrol inhibits the diaminoxidase activity of cow blood. The already noted [2] inverse relationship between concentration of the substrate and degree of enzyme inhibition must be stressed; this is quite consistent with the concept of a competitive nature of this inhibition.

In pig blood dimedrol enhances the activity of diaminoxidase; in this case too a definite relation can be observed between the concentration of the substrate and the effect of dimedrol; the higher the histamine content of the reaction mixture the less marked the enhancement of enzyme activity.

TABLE 1

Reaction Velocity Constants for Destruction of Histamine by Blood Diaminoxidase in Cows and Pigs

Concentration of histamine in γ/ml	Cow			Pig		
	K in control expts.	K in presence of dimedrol	changes in K under dimedrol influence in %	K in control expts.	K in presence of dimedrol	changes in K under dimedrol influence in %
10	0.007	0.005	−28.6	0.024	0.032	+33.3
30	0.0095	0.008	−15.8	0.029	0.035	+20.7
100	0.007	0.0075	+7.1	0 03	0.031	+3.3

The data presented in Table 1 suggest that the different character of the dimedrol effect on changes in diaminoxidase activity in cow and in pig blood depends on differences in the initial activity of this enzyme. Table 1 does in fact show that the diaminoxidase activity of pig blood is considerably higher than that of cow blood.

TABLE 2

Effect of Dimedrol on Reaction Velocity Constants for Destruction of Histamine (in 0.001M concentration) in Pig Blood Depending on Concentration of the Enzyme

Blood dilution	Reaction velocity constants for destruction of histamine		
	K in control expts.	K in presence of dimedrol (con. 0.001 M)	changes in K under dimedrol influence in %
Undiluted	0.0090	0.0122	+35.5
1:2	0.0071	0.0106	+49.3
1:4	0.0042	0 0063	+50
1:8	0.0038	0.0031	−18.4

If the character of the dimedrol effect on diaminoxidase activity depends on the initial activity of the enzyme it can be postulated that on dilution of pig blood, i.e., on lowering the enzyme concentration in it, the effect of dimedrol on the enzyme activity will approach its effect on the diaminoxidase activity of cow blood. This hypothesis was tested in special experiments whose results are presented in Table 2.

TABLE 3

Effect of Thiamine on Reaction Velocity Constants for Histamine Destruction in Pig Blood Depending on Concentration of the Enzyme

Blood dilution	Reaction velocity constants for histamine destruction		
	K in control expts.	K in presence of thiamine (concentration 0.0025 M)	changes in K under thiamine influence in %
Undiluted	0 009	0.0103	+14.4
1:2	0.0071	0.0051	−28.2

Table 2 shows that on considerable dilution (1 : 8) of pig blood the diaminoxidase activity is lowered under the influence of dimedrol.

According to literature data [4] thiamine is also an inhibitor of diaminoxidase. Experiments were carried out in which the effect of thiamine on the diaminoxidase activity of pig blood was examined. The results of these experiments are presented in Table 3.

As can be seen from Table 3, experiments with thiamine reveal the same relationship which was noted in the experiments with dimedrol: when the initial activity of diaminoxidase is high (undiluted blood) thiamine enhances its activity, when the initial activity of the enzyme is lower (2-fold dilution of blood) thiamine exerts an inhibitory effect on its activity.

This effect is consistent with the competitive concept of the relation between histamine on the one hand and dimedrol and thiamine on the other. As V. M. Karasik [1] has shown on a number of examples, pharmacologic reactions of the competitive type may reveal not only antagonism between the competing agents but also a phenomenon in which the pharmacologic agent foreign to the organism ("heterohaptone" according to Karasik) promotes interaction between the physiologic agent ("homohaptone") with which it is in a competitive relationship, and the corresponding reactive systems of the body. V. M. Karasik has emphasized the similarity of this phenomenon to the so-called "Haldane paradox" [3]. This paradox is known to consist of the fact that at sufficently low partial pressures of oxygen and carbon monoxide inadequate to ensure "saturation" of hemoglobin CO promotes the interaction of hemoglobin with O_2.

It can be visualized that the increased diaminoxidase activity observed under the influence of such agents as dimedrol and thiamine fits into the concept of the "Haldane paradox." In fact both dimedrol and thiamine promote the interaction between diaminoxidase and histamine only in those cases in which the initial activity of the enzyme is sufficiently high, i.e., when it can be assumed that there is a quantity of the enzyme which is not "saturated" by the substrate and the agent competing with it. Conversely, when the initial activity of diaminoxidase is lower and the enzyme can be assumed to be "saturated" completely by the added histamine the agents competing with it – dimedrol and thiamine – hinder the interaction between histamine and the enzyme in the same way as CO interferes with the formation of oxyhemoglobin under comparable conditions.

SUMMARY

The activity of blood diaminoxidase is changed under the influence of dimedrol and thiamine, i.e., its activity is either inhibited or, on the contrary enhanced.

It was established in experiments on pigs and cows that different action of dimedrol and thiamine depends on the initial activity of the enzyme. Thus, when the initial activity of diaminoxidase was high thiamine enhanced its activity, while with low initial activity of the enzyme the latter was inhibited by thiamine. The same principle was also experimentally proved in case of dimedrol. The mechanism of increase of diaminoxidase activity under the influence of dimedrol and thiamine is to a certain extent similar to the phenomenon of the "Haldane paradox."

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DEPENDENCE OF THE PHAGOCYTIC REACTION OF LEUKOCYTES ON THEIR CARBOHYDRATE METABOLISM

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N. V. Puchkov's work [1, 2] has shown that introduction of small concentrations of adrenalin into the animal's blood stream enhances the phagocytic activity of the blood. Since adrenalin is involved in the regulation of carbohydrate metabolism a study of the dependence of the phagocytic activity of the blood on the state of carbohydrate metabolism within the phagocytes was undertaken at this Department.

EXPERIMENTAL METHODS

A suspension of microbes prepared from a 1-2 day culture of Staphylococcus albus in dilution of half a billion per 1 ml was placed into a special test tube; the suspension was drawn up into a white-cell mixing pipette to the mark 1 and a similar amount of Ringer solution was added (in experiments with addition of glucose, insulin and other substances these were introduced in Ringer solution); blood taken from the finger was then added to the mixture. The test tube was then agitated carefully by rotary movement around its axis until a uniform mixture was obtained and the test tube was then placed in a thermostat for 30 minutes (phagocytic time). After 30 minutes a smear was made from the contents of the test tube and fixed in a mixture of alcohol and ether. The smears were stained by the Romanovskii method; the counts were always made along the edge of the smear on both sides. One hundred leukocytes – neutrophils – were counted. Among these, those that had behaved as phagocytes (positive) and those that had not (negative) were noted. The total number of positive leukocytes in relation to the total number counted (100) indicates the extent of phagocytosis.

It must always be borne in mind when investigating phagocytosis that the phagocytic reaction is not a constant entity and depends on many causal factors not always perceptible. In order to establish the degree of variability of this reaction under identical conditions a series of experiments was staged in which the phagocytic reaction of blood leukocytes was determined without any extraneous influences. Two test tubes were prepared simultaneously with the same blood (K_1 and K_2) for this purpose.

Twenty control experiments on 40 blood smears were carried out. The range of variation in the values was ± 8-10%. Therefore figures deviating from the control values by more than 10% could be taken as true deviation. In addition each experiment was accompanied by its own control.

EXPERIMENTAL RESULTS

Simultaneous addition of 1.6 international units insulin and 5% solution of glucose to the blood (25 experiments) leads to a sharp increase in the number of phagocytic leukocytes and also to enhanced phagocytic activity of the leukocytes which far exceeds the limits of chance variation (Fig. 1a). The average increase of the phagocytic index was 32%.

Experiments with separate addition to the blood of glucose (10 experiments) and of insulin (10 experiments) (Fig. 1, b and c) gave a directly converse result. When glucose alone was added the percentage of leukocytes showing phagocytic activity diminished by an average of 18-20%; insulin alone decreased phagocytosis to a still greater degree – by almost 25%.

Another method of affecting the carbohydrate metabolism of leukocytes was used in our experiments (14 experiments) and consisted of addition of monoiodoacetic acid in 1 : 10,000 dilution to the blood, i.e., interfering with glycolysis in the leukocytes (Fig. 2, b). It was found that abolition of glycolysis lowered phagocytosis by an average of 18-20% but did not pervent it completely.

Since lactic acid is a product of glycolysis it could be expected that its addition to phagocytes poisoned with monoiodoacetic acid would restore their normal activity. This proved to be in fact the case: when 40 mg% lactic acid was added to the blood simultaneously with the monoiodoacetic acid the phagocytic activity remained practically unchanged (Fig. 2a).

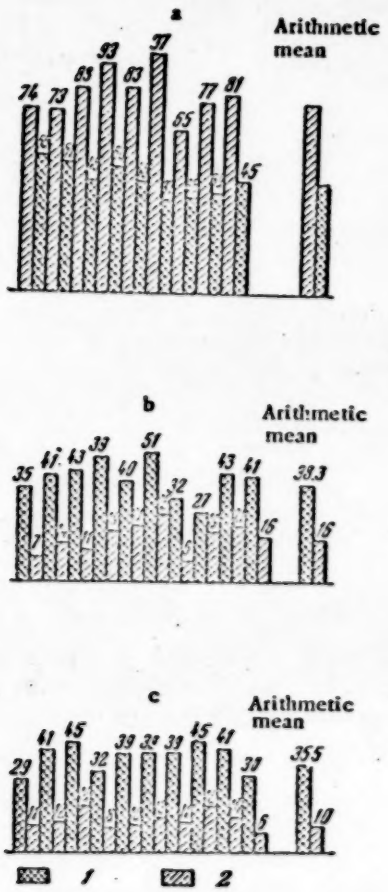

Fig. 1. Effect of insulin and glucose on phagocytosis. a) Addition of 1.6 international units insulin and 5% glucose solution; b) addition of 5% solution of glucose; c) addition of 1.6 international units insulin; 1) control; 2) experiment.

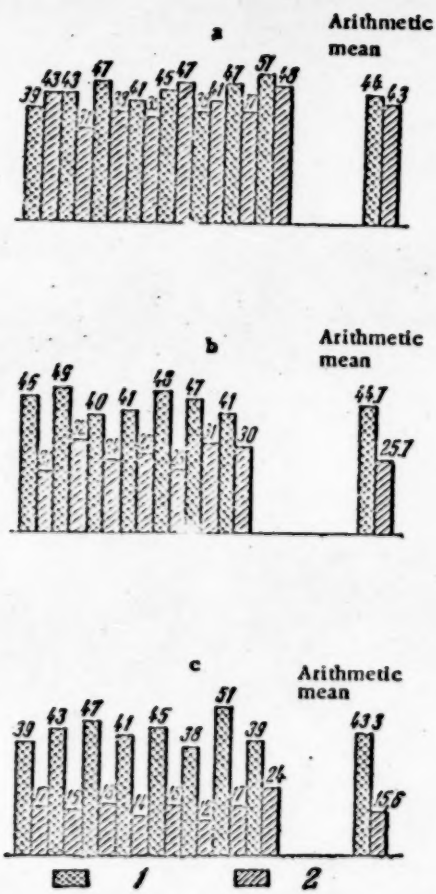

Fig. 2. Effect of monoiodoacetic acid and lactic acid on phagocytosis. a) Addition of 40 mg% lactic acid and monoiodoacetic acid in 1::10,000 dilution; b) addition of monoiodoacetic acid in 1 :10,000 dilution; c) addition of 40 mg% lactic acid; 1) control; 2) experiment.

For the purposes of control, the effect of lactic acid alone was investigated, using the same concentration (9 experiments) (Fig. 2b). Under these conditions there was an extremely marked drop in phagocytic activity — by an average of 20%.

In order to exclude the possibility that the changes in phagocytic index obtained were determined by a shift in pH the latter was checked electrometrically. It was found that the addition to the blood of these substances in the concentrations used in our experiments left the pH practically unaltered with variations within the limits of 7.5 to 7.8.

The data presented indicate that phagocytic activity depends to a considerable extent on the carbohydrate metabolism of the phagocytes; in other words, carbohydrates are an important source of energy required for the performance of this function.

SUMMARY

Glucose and insulin added together to the media causes a considerable increase of phagocytic activity of leukocytes of the human blood. Glucose and insulin added separately decrease this activity. Elimination of glycolysis induced by the addition of monoiodoacetic acid, likewise diminishes phagocytosis; this effect is abolished by the addition of lactic acid. The phagocytic activity of leukocytes depends to a great extent on their carbohydrate metabolism.

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PHARMACOLOGY

THE ANTISHOCK EFFECT OF AMINAZINE AND MEPAZINE

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Artificial hibernation is now being used successfully in clinical prophylaxis and treatment of shock.

The main components in the mixtures which are used to achieve a state of hibernation are derivatives of of the phenothiazine group of compounds. It is suggested that these compounds have the property of increasing the organism's resistance to trauma by lowering the intensity of metabolic processes. However, it is difficult to form an opinion of the strength of antishock effect of phenothiazine derivatives on the basis of clinical data only, since usually a whole complex of antishock measures is employed. Therefore, experimental investigations of the antishock effect of the compounds mentioned, tested against models of shock-like conditions, are of considerable interest. As shown by L. Beck and T. Redick [2], R. Milligan and E. F. Stohlman [5], preliminary administration of chlorpromazine lowers the percentage of white mice dying from tourniquet shock. Similar data were obtained by S. Courvoisier [4] who subjected white rats to traumatic shock.

The present work is devoted to the study of the antishock properties of mepazine – one of the new derivatives of the phenothiazine series, synthesized by S. V. Zhuravlev and collaborators at the Institute of Pharmacology and Chemotherapy, AMN SSSR. The antishock properties of aminazine were also studied for comparison.

The antishock properties of these preparations were compared with the similar effect of morphine as a substance used extensively in the prophylaxis and treatment of traumatic shock, both experimentally and clinically.

EXPERIMENTAL METHODS

The Noble and Collip [6] model of traumatic shock was used in the experiments but modified for white mice. The animals, with their limbs bound, were placed in a special drum 60 cm in diameter and 10 cm long. Within this drum two three-faceted projecting prisms were placed at diametrically opposite points on its circumference. The drum was rotated with the help of a synchronized electric motor with a speed of 30 rpm. During rotation the mice fell from one prism to the base of the other one which again lifted them upward from where they again fell to the opposite prism and so on. Not more than two animals were placed in the drum at the one time. As the result of these falls the mice developed a shock-like state and if rotation was continued they died.

According to the data available in the literature, the disturbances which arise in the animals as the result of such repeated falls within the drum resemble in many respects the disturbances observed in traumatic shock in man. Thus, R. Chambers and B. Zweifach [3] found that in the case of white rats 650-800 falls were followed by a drop of blood pressure to 30-40 mm Hg, the body temperature dropped from 96-100° Fahrenheit to 93-95°, the animals were extremely depressed, lay motionless, did not respond to pain stimuli, their skin showed pallor. Investigation of the capillary circulation showed that as the result of trauma there was engorgement of the intestinal blood vessels and constriction of the cutaneous ones. The total plasma volume dropped, as a rule, by 12-15% as compared with the initial level. The pathologic changes mentioned indicate that the the described condition of the animals can be considered as a model of traumatic shock.

A total of 16 series of experiments has been carried out on 320 mice. The mice were subjected to "rotation" in the drum until they died; the number of falls after which each mouse died was recorded. The data obtained were used to plot a curve of dependence of death (in percentages) on the degree of trauma.

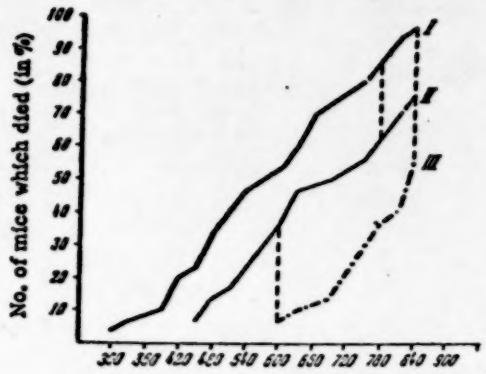

Fig. 1. Relation of death among mice and the degree of trauma in experiments with mepazine (II) and morphine (III), control (I).

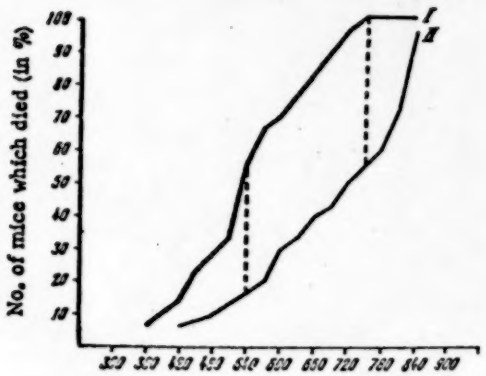

Fig. 2. Relation of death among mice and degree of trauma in experiments with aminazine (II), (I) control.

Control experiments showed that the relation of the animals' death to the degree of trauma was expressed by a fairly constant value and could be established even in experiments with relatively small numbers of mice.

Mepazine, aminazine and morphine were injected intraperitoneally 30 minutes prior to the infliction of trauma.

EXPERIMENTAL RESULTS

The experiments showed that mepazine and aminazine in the dose of 1 mg/kg body weight lowered the percentage of deaths among the mice. The most effective action of these preparations was observed when the trauma was of moderate degree (Fig. 1, 2). A similar relation between the antishock effect and the degree of trauma was also noted when other pharmacologic compounds were used [7].

The curves show that following administration of mepazine the number of mice which died from trauma of moderate degree dropped to 20%; it dropped by 45% when aminazine was given. Statistic treatment of the data obtained by the "chi-square" method showed that the difference between the mean results of the experiments and controls was significant.

Increasing the doses of mepazine and aminazine to 5-10 mg/kg did not raise survival among the mice — a reverse effect could even be observed. Similar data were obtained by Courvoisier in experiments with chlorpromazine on white rats [4]. Apparently the higher doses of these preparations lead to predominance of their toxic effect over their antishock properties.

Experiments with morphine gave results of a similar nature. When small doses of the preparation (1 mg/kg) were given the number of animals which died fell by 42% as compared with the control, but when higher doses were used the number increased rather than decreased. The greater effectiveness of small doses of morphine has also been noted by clinicians [1].

SUMMARY

Aminazine (Chlorpromazine) in doses of 1 mg per kilogram of body weight decreases the death of white mice from traumatic shock by 45%, mepazine by 20%, morphine by 42%. Higher doses of these preparations do not increase the percentage of survival of these animals.

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THE COMPETITIVE CHARACTER OF THE ACTION OF GANGLION BLOCKING AGENTS – DERIVATIVES OF QUATERNARY AMMONIUM COMPOUNDS

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Despite the large number of experimental and clinical investigations of substances which inhibit synaptic transmission of nerve impulses, the question of the mechanism of their action has not as yet received adequate study. The majority of authors tend to explain the pharmacologic activity of these compounds by competitive antagonism because anticholinergic preparations, and ganglion blocking substances in particular (quaternary ammonium derivatives) approach in their chemical structure acetylcholine, a mediator substance [1, 3, 10, 11]. In studying the so-called methonium series of ganglion blocking agents we were also led to the conclusion that their central and peripheral and anticholinergic effects could be explained on the basis of competitive interrelations with acetylcholine [8]. It follows from this that anticholine esterase preparations, which stabilize acetylcholine, should also enter into competitive relations with ganglion blocking agents when acting on cholinergic structures.

Investigations by M. Ia. Mikhelson and collaborators [4] are significant; these authors established that the central effects of the anticholinergic substance pentaphen could be blocked by means of the anticholine esterase preparation proserine. Recently M. M. Kholodenko [9] attempted to make a similar study with respect to anticholinergic substances of the quaternary ammonium derivative type. However, the method used by that author was not without defects. The tests used in his work – the cholinergic complex and hypertension on administration of eserine as well as mortality of the animals made it impossible to evaluate the observed phenomena uniformly, since these phenomena were a consequence of numerous functions of the body; therefore the subsequent administration of ganglion blocking agent by the method adopted by the author could not demonstrate the mechanism and localization of the antagonistic action of the given substances with sufficient conviction.

EXPERIMENTAL METHODS AND RESULTS

The experimental conditions for demonstration of the interrelations in the system acetylcholine-ganglion blocking agent-anticholine esterase preparation consisted of excitation of cholinergic structures by adequate stimuli with subsequent use of the ganglion blocking agent. In the second series of experiments, whose results were compared with those of the first, stimulation of one or other cholinergic structure was combined with the use of the anticholine esterase proserine; the ganglion blocking agent was then tested against this background. The most typical representative of the quaternary ammonium bases – hexamethylene-bis-trimethyl ammonium iodide (synonyms: hexamethonium, hexonium, hexatonide) • – was used as the ganglionic blocking agent.

In order to find out how universal the principle of competitive antagonism might be, various cholinergic structures affected by the specific action of hexonium were selected as objects of investigation. Such structures included ganglionic N-cholinergic structures of the vagus nerve ganglia in cats, N-cholinergic structures of skeletal musculature (frogs), N-cholinergic structures of the central nervous system (frogs) and peripheral M-cholinergic structures (mice).

• The preparation was synthesized at the Department of General Chemistry of the Chernovits Medical Institute by Assistant Professor A. I. Lopushanskii.

Fig. 1. Abolition of the ganglionic blocking effect of hexonium by proserine. Experiment performed on cat. Records from above down: blood pressure response to electric stimulation of preganglionic fibers of the vagus, stimulus marker, time marker (5 seconds).

Peripheral N-cholinergic structures. In the first series of experiments the relation between hexonium and proserine in their effect on N-cholinergic structures of the cardiac ganglia of the vagus was studied. Experiments (10) were performed on cats under urethane anesthesia or on decerebrate preparations. Hexonium was injected into the femoral vein in doses of 0.1-5 mg/kg. The ganglion blocking action of the compound was judged by the hypotensive reaction when the preganglionic fibers of the vagus were stimulated by induction current of suprathreshold strength. Control experiments established that hexonium in doses of 1-5 mg/kg produced constantly complete and prolonged (up to 30 minutes and longer) ganglionic blocking effect.

Preliminary intravenous injection of proserine in doses 0.01-0.025 mg abolished the ganglion blocking effect of hexonium (Fig. 1).

The curare-like action of hexonium was used for the study of competitive interrelations on N-cholinergic structures of skeletal musculature. Frogs were used in these experiments. The results of the experiments were recorded on kymograms.

The first series of investigations was carried out on the hind limbs of frogs prepared by Pisemskii's method (7 experiments). Perfusion with a hexonium solution of 1×10^{-4} concentration evoked progressively increasing curare-like effect which was manifested in a sharp decrease of reaction to stimulation of the peripheral end of the sciatic nerve by single shocks of induction current of suprathreshold strength at the rate of one shock per minute. Subsequent perfusion with proserine in 1×10^{-4} concentration was accompanied by restoration of the amplitude of contraction in the frog's leg.

In the second series of experiments (9 experiments) the isolated frog rectus abdominis was used. Hexonium in concentration of $3 \times 10^{-3} - 3 \times 10^{-6}$ caused relaxation of acetylcholine-induced contraction (acetylcholine in concentration of 3×10^{-7}). Administration of proserine in concentration of 1.5×10^{-6} protected the muscle from relaxation (Fig. 3). It is characteristic that higher concentrations of hexonium again exerted a curare-like effect.

Peripheral M-cholinergic structures. We could find no literature references to the effect of ganglion blocking substances of the quaternary ammonium derivative type on peripheral M-cholinergic structures. Nonetheless it appeared interesting to attempt to obtain a blocking effect on these structures since here too the competitive interrelations under consideration could, presumably, take place.

Experiments were carried out on 28 white mice weighing 18-30 g. In control experiments subcutaneous injection of 5-6 mg acetylcholine was accompanied by increased salivation, micturition and defecation. All these manifestations were easily abolished by preliminary or subsequent subcutaneous injection of small doses of atropine (0.02-0.04 mg) which proved the peripheral character of the observed phenomena.

Like atropine, hexonium given subcutaneously in doses of 0.2-0.4 mg 10 minutes prior to acetylcholine caused marked diminution, and in most cases complete disappearance, of the manifestations mentioned above. Preliminary administration of 0.05-0.5γ proserine with subsequent administration of hexonium and acetylcholine in appropriate doses restored the original picture of acetylcholine intoxication in mice.

Central N-cholinergic structures. Manifestations of competitive antagonism occurring in central N-cholinergic structures were studied in two series of experiments. The first series made use of the property of this group of ganglion blocking preparations of preventing the specific reaction of frogs to nicotine (this property was established earlier by us [7]), the central character of this reaction having been proved by many authors [2, 5, 6].

Experiments were performed on 24 Rana esculenta frogs weighing 40-50 g. Preliminary experiments established that hexonium in doses of 8-10 mg blocked the characteristic nicotine reaction (1 mg nicotine) completely or partially. Preliminary (5-10 minutes earlier) administration of proserine in doses of 0.5-0.75 mg with subsequent administration of hexonium and nicotine in the doses indicated above was not accompanied by any substantial changes in the reaction of the frogs to nicotine.

In the second series consisting of 12 experiments, the blocking effect of hexonium on central N-cholingergic structures was studied on spinal forgs with determination of the latent period of the Turk flexor reflex. The experimental results were recorded on kymograms.

It was found that introduction of 2-4 mg hexonium into the dorsal lymph sac of the frog caused a considerable (2-4 fold) increase of the latent period for the reflex. Preliminary administration of proserine in the dose of 0.5 mg abolished this effect of hexonium.

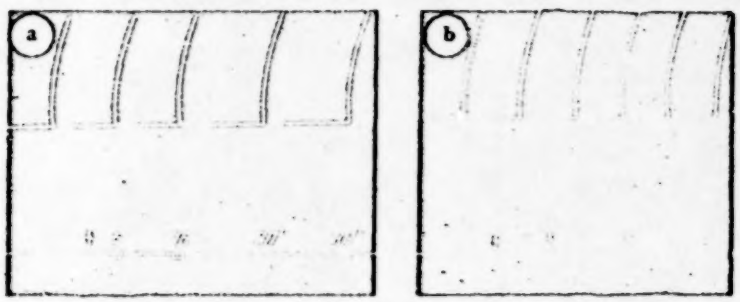

Fig. 2. Effect of hexonium on the length of the latent period of the flexor reflex according to Turk (a) and abolition of this effect by proserine (b). In both traces the stimulus marker shows the latent period for the reflex.
↑ – hexonium, 4 mg; ↑↑– proserine, 0.5 mg.

Since prolongation of the latent period of the reflex could have been a consequence of changed functional state of the efferent part of the reflex arc, the same frogs were subjected to stimulation of the peripheral end of the sciatic nerve by single shocks of induction current of suprathershold strength and the contraction of the hind limb muscle in response to this stimulation was determined. Administration of hexonium did not alter the observed reaction appreciably. In other words, hexonium given in the doses indicated and by the route employed did not exert a curare-like effect and its influence on the duration of the latent period of the reflex was apparently determined by the blocking of the central portions of the reflex arc.

The experimental material obtained confirms the postulated hypothesis concerning the competitive character of phenomena occurring at cholinergic structures under the influence of the ganglionic blocking substances of the group under consideration. If the extensive functions of acetylcholine in the transmission of impulses in the interneuronal synapses of the central and peripheral components of the reflex arcs are taken into account,

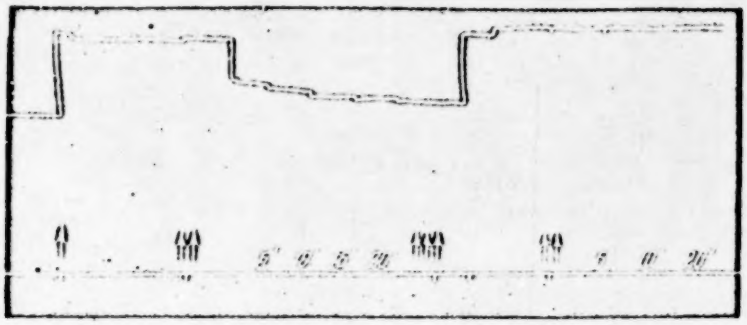

Fig. 3. Abolition of the curare-like effect of hexonium by proserine. Experiment on frog rectus abdominis preparation. ↑ – acetylcholine, 3×10^{-7}; ↑↑ – hexonium, 6×10^{-5}; ↑↑↑ – proserine, 1.5×10^{-6}.

it becomes clear why the stabilizing influence of the anticholine esterase preparation proserine inhibits various manifestations of the anticholinergic action of hexonium which approaches acetylcholine in its structure.

It appears to us that further investigations directed towards the study of the intimate mechanisms of action of anticholinergic preparations of this series can be of use in searching for therapeutic substances capable of normalizing the nervous regulation of various functions.

SUMMARY

An attempt was made to prove experimentally the competitive character of the ganglion blocking preparations – derivatives of the quaternary ammonium compounds. The stabilizing effect of proserine on the mediator function of acetylcholine was used for that purpose. It was established by these experiments that proserine inhibits the blocking effect of hexonium (which is very similar in structure to acetycholine) on the various peripheral and central N and M cholinergic structures.

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MICROBIOLOGY AND IMMUNITY

A STUDY OF THE BACTERIOSTATIC AND CHEMOTHERAPEUTIC ACTIVITY OF THE NEW DRUG CYCLOSERINE

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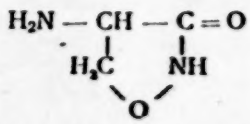
In 1956, in the department of organic synthesis of the Institute of Pharmacology and Chemotherapy of the AMN SSSR, Prof. N. K. Kochetkov and a group of his co-workers – M. Ia. Karpeiskii, R. M. Khomutov and and N. F. Kucherova [2, 3] synthesized a new chemotherapeutic drug – dl-cycloserine or 4-aminoisoxazolidone – 3.



In the present article we give the results of a study of the bacteriostatic and chemotherapeutic activity of cycloserine.

EXPERIMENTAL METHODS AND RESULTS

We tested cycloserine by experiments both in vitro and in vivo. The antibacterial activity was studied in a liquid broth medium to which the drug was added in increasing concentrations, starting with 2 γ /ml and rising to 1000 γ/ml. The bacterial load in all cultures amounted to 250,000 microorganisms per 1 ml of medium.

The experimental results are shown in Table 1.

As shown by Table 1, cycloserine has a broad spectrum of activity. It is active against Gram-positive and Gram-negative microorganisms such as B. typhi abdominalis 4446, B. paratyphi A, B. paratyphi B, and also against various species of dysentery bacilli.

The action of cycloserine against Mycobacterium tuberculosis was studied in Soton's synthetic liquid medium with the addition of 10% human plasma. The following tuberculosis cultures were used in the experiment: a laboratory culture of B. bovinus 8, three freshly isolated cultures of the human type 123, 126, 122 and strain BCG. Strain 126 was sensitive to streptomycin, phthivazid and PAS; strain 123 was resistant to 100 units of streptomycin, 10 γ/ml of phthivazid and 50 γ /ml of PAS; strain 122 was sensitive to streptomycin and PAS and resistant to 10 γ/ml of phthivazid. For each strain which we tested we set up 3 experiments with 2 different bacterial loads in each: 27 million microorganisms per 1 ml of medium and 5.5 million microorganisms per 1 ml of medium. Readings were taken every 7 days, starting at the end of the first week, when

the control culture showed a good growth. The results, which are given in Table 2, are the mean values of the 3 experiments.

TABLE 1

Activity of Cycloserine Against Various Microorganisms

Serial No.	Culture	Activity in γ/ml
1	Staphyl. 209	62.5
2	Staphyl. Wood	62.5
3	B. coli 613*	31.2
4	B. coli 35	31.2
5	B. coli (resistant)	Not active
6	Proteus OX_{19}	250
7	B. pyocyaneum	250
8	Sarcina lutea	3.1
9	B. subtilis	250
10	B. mycoides P-20	31.2
11	B. typhi abd. 4446	15.7
12	B. paratyphi A 299	125
13	B. paratyphi B 42	31.2
14	B. dys. Flexneri	31.2
15	B. dys. Sonne 714	31.2

* Primary culture.

As may be seen from Table 2, cycloserine possesses a bacteriostatic action against Mycobacterium tuberculosis, but this activity is not high and is inferior to that of streptomycin, phthivazid and PAS. The sensitivity of different tuberculosis cultures to cycloserine is not the same. The culture bovinus 8 was found to be most sensitive to the drug, and experimental virulent strain.

It must be mentioned that cycloserine is active also against freshly isolated human strains which are resistant to streptomycin, phthivazid and PAS. Dynamic observations show the necessity of higher concentrations of cycloserine to suppress the growth of the tuberculous culture during the later stages. For this reason we set up special experiments to study the inactivation of the drug. As a result of these experiments it was clear that on standing on an incubator at 37°C for 8-10 days, cycloserine loses about 25-30% of its initial activity.

We investigated the chemotherapeutic activity of the drug in white mice with hematogenous tuberculosis and guinea pigs with tuberculosis.

For the experiment we used white mice weighing 18-20 g. The animals were infected intravenously with 0.1 mg of the culture of bovinus 8.

TABLE 2

Activity of Cycloserine Against Mycobacterium Tuberculosis

Strains	Bacterial load in millions of microorganisms per 1 ml of medium							
	27				5,5			
	readings taken on days							
	7	14	21	23	7	14	21	28
	activity in γ/ml							
B bovinus 8	3.26	6.8	13.9	13.9	No growth	3.5	13.9	13.9
Human type 123	6 6	17.5	24.0	—	"	8.8	22.2	—
" 126	16.6	41.6	55.5	—	8.8	22.2	—	—
" 122	9.7	14.9	27.7	—	No growth	7.7	16.6	—
BCG	4.1	7.7	14.7	—	"	3.2	11.1	—

Treatment began the day following infection, a dose of 0.5 mg of cycloserine per mouse being given orally. The control and treated groups each comprised 15 mice. The results of the experiments were assessed by the macroscopic changes in the lungs, the weight of the lungs and the number of tubercle bacilli in films taken from the lungs. Macroscopic changes in the lungs and the number of bacilli in the lung films were dedetermined by the three-point system proposed by M. V. Trius [4]. In Table 3 are given the mean indices for both groups of animals.

It follows from Table 3 that the drug does not show any very marked chemotherapeutic activity, although it must be noted that all the animals in the treated group were alive up to the end of the experiment. Some

Some variation is also observed in the indices of the macroscopic and bacterioscopic changes in the lungs. Of the 15 control animals, seven died between the 21st and 29th days, while all the mice of the treated group survived and were killed with the remaining living control mice on the 30th day.

The experiment to study the chemotherapeutic activity of cycloserine on guinea pigs with experimental tuberculosis was performed on 60 animals weighing 220-250 g, infected subcutaneously in the region of the right groin with 0.0001 mg of a culture of bovinus 8.

TABLE 3

Index of the Macroscopic Changes, the Weight of the Lungs and the Number of Tubercle Bacilli in Films of the Lungs in Treated and Untreated Mice

Animals	Mean index of macroscopic changes in the lungs	Mean weight of the lungs in g	Number of tubercle bacilli in films from the lungs (index)
Treated	1.9	530	1.6
Untreated (control)	2.3	568	2.6

According to the form of the treatment we divided the guinea pigs into 5 groups: groups I and III were treated with cycloserine at the rate of 5 mg orally once daily; groups II and IV were given 30 mg orally once daily; group V was the control. Treatment in groups I and II started 2 weeks after infection; in groups III and IV, 5 weeks after.

The following observations were made on the animals during the experiment: weight, inspection of reaction at the site of infection and in the regional lymphatic glands and tuberculin tests. All the animals were killed on the 117th day after infection. Macroscopic changes found at necropsy were assessed according to the system of Iu. K. Veisfeiler [1].

TABLE 4

Index of the Macroscopic Changes in the Lungs of Guinea Pigs

Group of animals	Treatment	Mean index of macroscopic changes
I	5 mg 2 weeks after infection	16
II	30 mg 2 weeks after infection	16,1
III	5 mg 5 weeks after infection	16,5
IV	30 mg 5 weeks after infection	17
V (control)		15,3

From observations on the animals during the experiment no difference was observed in either the time of appearance of tuberculin sensitivity or in the change in the weight of the treated and untreated animals. As is seen from Table 4, the degree of the macroscopic changes in the animals of the different groups was also the same.

Thus under the conditions of this experiment, cycloserine is ineffective in the treatment of experimental tuberculosis in guinea pigs.

The results show that cycloserine has low toxicity towards guinea pigs, since the animals which were infected with tuberculosis tolerated well doses of the order of 30 mg per guinea pig (100-150 mg) over a prolonged period – about 100 days.

As we know, D-cycloserine obtained in the U.S.A. by a biological method is active against Mycobacterium tuberculosis in vitro [5, 9] but is not active in the treatment of experimental tuberculosis in mice and guinea pigs [7, 10]. Nevertheless D-cycloserine has been found to be a valuable drug in the treatment of various forms of tuberculosis in man [6, 8]. The reasons for this discrepancy between its chemotherapeutic activity in vitro and in vivo are not yet known. On the basis of these findings it is worth trying dl-cycloserine in hospital practice for the treatment of tuberculosis in man.

SUMMARY

Synthetic dl-cycloserine obtained at the Institute of Pharmacology and Chemotherapy of the USSR Academy of Medical sciences was tested in experiments in vitro. It was demonstrated that this preparation has a wide antimicrobic range of action towards gram positive, as well as towards gram negative microbes, such as B. typhi abdominalis, B. paratyphi "A" et al.

Dl-cycloserine has a bacteriostatic effect toward various strains of mycobacterium tuberculosis which are sensitive or resistant to streptomycin, phthivazid and PAS.

It was revealed that this preparation does not show any significant chemotherapeutic effect in experimentally indiced hematogenic tuberculosis in mice and tuberculosis in guinea pigs. However, its toxicity in prolonged administration to guinea pigs is very low. If kept in a thermostat during 8-10 days at a temperature of 37°C the preparation is inactivited by 25 to 30%.

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THE DETECTION OF TISSUE ANTIGENS

REPORT NO. III. THE COMPARATIVE SENSITIVITY OF THE ANAPHYLAXIS REACTION AND SEROLOGICAL INVESTIGATION IN THE DETECTION OF SMALL DOSES OF ANTIGEN

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The proposal to use the reaction of anaphylaxis to detect the individual components of the antigenic complex is based, as we know, on the idea that it is more sensitive than serological methods. However, as was shown in previous reports [1, 2] the use of the anaphylacitc reaction for this purpose is beset with serious difficulties and a number of conditions must be rigidly complied with. Further, the great improvements which have taken place in recent years in serological methods of investigation must be borne in mind. The question of comparison of the two methods naturally arose, in order to determine whether and in what conditions the anaphylactic reaction was more sensitive than the serological examination, and whether the level of sensitivity of the latter could be increased to that of the former.

This question could be answered by comparing the minimum dose of antigen to which guinea pigs sensitized passively with varying amounts of immune serum would react with the dose of antigen which could be detected by the same serum in experiments in vitro.

EXPERIMENTAL METHODS AND RESULTS

Guinea pigs were injected intravenously with immune serum to horse albumin in different doses from 0.3 to 0.002 ml per 100 g body weight; the corresponding possible (maximum) content of immune serum in the body of the animals in the different groups was calculated. In order to produce an anaphylactic reaction, antigen (horse serum) was injected (24 hours after injection of the immune serum) also in different quantities, so that by calculation in relation to the weight of the animals its concentration in the body varied from 1:500 to 1:160,000.

The results of the experiments are given in Table 1. It was shown that with the smallest dilution of immune serum (1:320) a clear anaphylactic reaction could be obtained if the antigen was injected in quantity to give a concentration in the body of the animal of 1: 80,000. As shown in Tables 1 and 2, at such a dilution the antigen is found very clearly also by an in vitro experiment by means of the complement fixation reaction with immune serum diluted 1: 100 and even 1:400. However a clear anaphylactic reaction was also observed when the antigen content of the animal was lowered (to 1:6400 and even to 1:256,000) if the quantity of antigen injected was increased and its final concentration in the body of the animal reached 1:10,000 or over. By the serological method it was not possible to detect the antigen in this dilution even when immune serum was injected up to a concentration of 1:1600 or over.

The results obtained showed that the great sensitivity of the anaphylactic reaction was due to the fact that it requires a relatively small quantity of antibody. This is presumably because the antigen-antibody complex formed is inadequate for adsorption of complement but may act as a stimulus to the animal. With an

immune serum of high titer and used in small dilutions, antigen may be detected by means of the serological method in the same minimal quantity as by means of the in vitro experiment. Unfortunately it is not certain that immunization with tumor material produces an immune serum with a high content of antibody to the tumor cells. This gave rise to the question whether it is not possible to increase the sensitivity of the seroreaction at this particular antibody titer. This would obviously require the introduction of a larger amount of antibodies into the reacting mixture, i.e., if the dilution of the serum is unchanged then it would have to be injected in larger volume. In fact, when this same immune serum which was used in the experiments described above was tested by the complement fixation reaction in a dilution of 1: 6400 but in ten times the volume of the antigen, a positive results was obtained with a high dilution of antigen (over 1 : 64,000). In this case the results of the in vitro experiment were not inferior to those of the passive anaphylaxis experiment in guinea pigs which had been sensitized by the same immune serum in doses of such a size that the concentration of antibody in the animals was equal to that used in the serological experiment (Table 3).

TABLE 1

Detection of Horse Albumin by Means of the Anaphylactic Reaction with Passive Sensitization

Concentration of antigen injected as the assaulting dose into the guinea pigs	Reaction of the animals in which immune serum had been injected to cause sensitization in an amount of:				
	1:320	1:3200	1:6400	1:12 800	1:25 600
1 : 500					+++
1 : 1000					+++
1 : 10 000		Died		++	++
1 : 20 000		+++	++(+++)		++
1 : 40 000		+(++)	+++	+(++)	
1 : 80 000	++(+++)	+			
1 : 160 000	—				

Meaning of symbols: +++ marked anaphylactic shock; ++ clear reaction with marked bronchospasm (spasmodic cough); + weak reaction: sneezing, scratching, repetitive movements, shortness of breath.

In view of these results it was important to discover if it is possible by immunization with a mixture of antigens in different quantitative proportions to obtain antibodies in adequate titer to the "minor" antigen and if antibodies to the "minor" antigen could be retained in the serum after removal of the antibodies to the "major" antigen.

For this purpose rabbits were immunized with a mixture of horse and rat sera, in proportions of 50: 1 and 250: 1. The total quantity of "minor" antigen (rat serum) injected was 0.04 ml per 1 kg body weight of the animals in the first group, and 0.008 ml for the second group; this was divided into 4 intravenous injections given at intervals of 4-5 days. Antibodies to rat serum were obtained in two of the three animals of the first group and in one rabbit out of three in the second group.

The content of antibodies in the blood in two pairs of rabbits which had been immunized with corresponding doses of rat serum alone was found to be higher. It should be mentioned that by using the method of a large volume of antibodies it is possible to increase considerably the sensitivity of the seroreaction. By this method, in experiments with sera obtained from rabbits immunized with a mixture of antigens, the importance was clearly shown of optimum proportions between the reacting components: the reactions were negative with a larger dose of antigen and positive at higher dilutions.

In another series of experiments rabbits were immunized with a mixture of so-called "weak" antigens, i.e., nucleoproteins from organs of the horse and rat in proportion of 50: 1 , so that the calculated quantity

of proteins of the "major" antigens injected was 32 mg and that of the "minor" antigens was 0.64 mg for the whole cycle of immunization (4 intravenous injections at intervals of 4-5 days). In this case antibodies to the "minor" antigen were detected (in dilutions of sera of 1:25 and antigen up to 1:100) in all 3 rabbits in the experiment, but at a lower titer than in animals immunized by the "minor" antigen alone in the same dose (in this case clear positive results were obtained also in serum dilutions up to 1:100 with antigen in dilutions of 1:100 and 1:200).

Investigation of the problem of preservation in the serum of antibodies to the "minor" antigen after removal of antibodies to the "major" antigen was made necessary by the fact that in experiments with soluble antigens we had to resort to a number of additional manipulations (dialysis of the sera and fractionation) which might affect the content of antibodies.

TABLE 2

Detection of Horse Albumin by the Complement Fixation Reaction with Various Dilutions of Immune Serum and Antigen

Dilution of antigen	Dilution of immune serum				
	1:100	1:400	1:800	1:1600	1:3200
1:100	++++	++++	−	−	−
1:5000	++++	++++	++++	−	−
1:10 000	++++	++++	++++	±	−
1:20 000	++++	++++	++++	−	−
1:50 000	++++	++++	+	−	−
1:100 000	+++	+	−	−	−

Meaning of symbols: ++++ total suspension of hemolysis with a double dose of complement; +++ partial suspension of hemolysis with a double dose and total suspension with 1½ doses of complement; ++ total suspension of hemolysis with 1½ doses of complement; + partial suspension of hemolysis with one dose of complement.

In setting up experiments with immune sera obtained by immunization with a mixture of two "potent" antigens, as a first step the dose of the "major" antigen was determined which was sufficient to completely extract the corresponding antibodies. Exhaustion of the sera was brought about by doses which were 4 and 10 times greater. It was found that antibodies to the "major" antigen were completely extracted and they could not be detected by the large volume method. At the same time antibodies to the "minor" antigen were retained in the serum, although in a somewhat reduced amount. In these cases it was possible by using the method of large volumes of immune serum to detect by means of these sera very small quantities of the "minor" antigen.

Less clear results were obtained in experiments with sera from rabbits immunized with a mixture of the so-called weak antigens. In order to achieve complete exhaustion of these sera from the "major" antigen (horse nucleoprotein) relatively larger quantities were required than in the similar experiments with sera against strong antigens (100-200 mg of protein against 20 ml for 1 ml of serum). After such treatment the reactions with the "minor" antigen were weak and not sufficiently clear.

DISCUSSION OF RESULTS

As shown by the results described, the superiority of the anaphylactic reaction over serological investigations lies in the fact that with it it is possible to detect antigen with a low concentration of antibodies, insufficient for giving a positive result in experiments in vitro. It is necessary to make use of the high sensitivity of the living reacting system but to reduce so far as possible the difficulties inherent in experiments in vivo, described in previous reports [1, 2]. For this purpose passive anaphylaxis could be tried, excluding desensitization, i.e., sensitizing guinea pigs with tumour antiserum from which the antibodies to normal tissues had been extracted. It is possible that in the presence of specific (tumor) antibodies they would prove adequate to

produce a reaction in the animal to injection of tumor material. This method would remove many difficulties; total exhaustion of the immune serum could be checked with sufficient accuracy; the animal would not be subjected to repeated and far from indifferent influences before the experiment; all controls would be clear and straightforward. It should be pointed out that pilot experiments to study this method gave positive results. However a detailed quantitative analysis is required, and this must be one of the next tasks to be undertaken in the investigation.

Furthermore the results of the experiments show, from the characteristic features of the serological method, that this method should not be abandoned in the study of the antigenic properties of tumors; on the contrary there are grounds for reckoning on increasing its sensitivity and accuracy of reading. Already two points can be mentioned which focus attention in this direction: 1) consideration must be paid to the law of optimum proportions, and the serological examination must be carried out with various doses of test material (the so-called "square" scheme); 2) the results obtained must be checked and supplemented by experiments by the method of large volumes of antibodies.

TABLE 3

Comparative Sensitivity of the Anaphylactic and Complement Fixation Reactions, Using the Method of a Large Volume of Antibodies

Experiment in vivo				Experiment in vitro			
concentration of antigen in the assaulting dose injected into the guinea pigs	content of immune serum in the guinea pigs with passive sensitization			dilution of antigen	dilution of immune serum		
	1:1500	1:1000	1:24000		1:1600	1:6400	1:25600*
1 : 10 000	Died	+++	+	1 : 16 000	++++	++++	++
		++(+++)	+	1 : 64 000	++++	++++	++
1 : 40 000	Died	++	+	1 : 256 000	++++	++	±
	+++	++	+	1 : 1 024 000	++	−	−
				1 : 4 099 000	−	−	−

Meaning of the symbols is the same as in Tables 1 and 2.

However it must be stated that the above-mentioned analysis related to sera which had been obtained by immunization of animals with a mixture of two different antigens. It is extremely important to perform similar experiments with a mixture of related antigens, i.e., possessing common antigenic groups. In the results quoted it was mentioned that exhaustion by a large amount of horse nucleoprotein (and subsequent treatment) extracted a large proportion of the antibodies to rat nucleoprotein from the sera of rabbits which had been immunized with a mixture of these two antigens in a proportion of 50: 1. Attention was directed to the fact that the sera of rabbits immunized against horse nucleoprotein alone, also reacted with antigen obtained from tissues of the rat although, naturally, much more weakly than with the homologous antigen. If this is on account of common components in the preparations used, then a careful study of the conditions in which it is possible to differentiate the individual antigenic components forming part of the common groups is an urgent future task in the investigation. This must first be undertaken in suitable experimental animals.

This careful study in suitable experimental animals also applies to new methods which have been introduced into serological research, such as: 1) replacement of the complement fixation reaction by the agglutination reaction with preliminary adsorption of antigen on the surface of cells or particles of a fine suspension, and 2) the precipitation reaction in a gel with counter diffusion of antigen and antibodies.

Finally we must deal with one aspect of principle, namely the character of tumor antigens and their corresponding antibodies. There would appear to be adequate grounds for considering that they belong to the group of autoantigens. In this case it must be remembered that autoantibodies may belong to the "incomplete" category and consequently require special methods for their detection. Is it not because there is so much that

is difficult, unexplained and even questionable about tumor antigens that we have so far failed to come near to a correct determination of their essential character and to study them ? Investigations in the light of modern ideas on autoantigens and autoantibodies are therefore of urgent importance.

SUMMARY

Sensitivity of the general methods was compared. For that purpose the following determinations were carried out: 1) the minimal dose of the antigen, to which guinea pigs, passively sensitized by various doses of the corresponding immune serum, reacted and 2) the quantity of the antigen which could be determined by the same immune serum in experiments in vitro. Anaphylactic reaction was found to be more advantageous in experiments with low doses of immune serum. Sensitivity of the serological method could be increased in conducting experiments by the so called, "rectangular" scheme, which takes the law of optimal ratios into consideration, as well as by using the method of large volume of antibodies. In order to reveal the components of the complex antigen one should study the method of passive anaphylaxis by sensitization of animals by immune sera, in which the antigens of the normal tissues were previously exhausted in vitro.

The problems which are next to be investigated are, the methods of differentiation of antigenic components which possess common antigenic groups, the characteristics of the new serological methods, the problem of tumor antigens with consideration of conceptions on autoantigens.

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* Original Russian pagination. See C. B. Translation.

THE EXCRETION OF DYSENTERY ANTIGENS BY THE KIDNEYS IN DOGS WITH UNCHANGED AND CHANGED IMMUNOLOGICAL REACTIVITY

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According to the latest findings, the mammalian kidney is permeable to macromolecular substances such as hemoglobin, serum and foreign proteins and toxins, antigens and the microorganisms of scarlet fever, croupous pneumonia, dysentery and typhoid fever.

Excretion of viruses in viral diseases was proved by L. A. Zil'ber [2], who also raised the question of the importance of this process in immunity.

It was shown by G. Wallenius [12] that the clearance index of the simplest macromolecules of the polysaccharide dextran was inversely proportional to the molecular weight. It was found that large-molecular substances undergo reabsorption in the tubules, as was shown in the case of protein marked with Evans blue and fluorescein [8, 11]. The question of the excretion of microorganisms by the healthy kidney has been examined even in the earliest stages of the development of immunology (V. Vysokovich, I. I. Mechnikov cited by [1, 2, 4], and later by L. Ascher and J. Sokol (cited by [1, 2, 4]). The majority of workers answered this question in the negative.

Excretion by the kidneys of bacterial antigens, toxins and viruses has been proved, and their removal may be regarded as one of the pathological mechanisms of immunity [1].

In the present work the excretion of dysentery antigens from the kidneys of dogs was studied during experimental dysentery poisoning.

EXPERIMENTAL METHODS

The investigation was made in chronic experiments on dogs. A constant infusion by the drip method of a 1% solution of inulin (or a 1% solution of thiosulfate) and simultaneously of Flexner dysentery antigens (prepared at the I. I. Mechnikov Institute, series 360) was set up. The antigens injected amounted to 0.1-0.4 mg per 1 kg body weight for 15-30 days. During this time complete clearance of antigens from the animals took place. After administration of water (40 ml per 1 kg body weight) to the dog, it was placed on a board, and one of its hind paws was fixed to the board with bandages. A needle, joined by a rubber tube to a vessel containing the solutions of substances to be injected, was inserted into the dorsal lateral metatarsal vein and fixed with two strips of adhesive plaster to the shaved or depilated surface of the paw.

In order to produce a definite concentration of the injected substances in the blood, their rate of flow was regulated by compression of the lumen of the tube with a rubber band.

The experiment consisted mainly of 2 clearance periods each of 20 minutes. The urinary bladder was emptied by catheterization or else the urine was collected through a Pavlov-Orbeli fistula. In the middle of the clearance period blood was taken from the dorsal lateral metatarsal vein on the side opposite to that in which the injections were being given.

The concentration of thiosulfate in the blood and urine was determined by an iodometric method, and the inulin colorimetrically by means of a thiourearesorcin reagent. Quantitative determination of dysentery antigens in the blood was carried out by means of the cold complement fixation reaction. The clearance index of the injected substances was then calculated. Further, the antibody titer in the blood was checked by the precipitation reaction and control analyses of the urine were made.

Experimental Results of Administration of Dysentery Antigen to Nonimmunized Dogs

Experiments were carried out on 8 dogs not previously immunized. In view of the fact that antigen was injected repeatedly into many of these animals, experiments in which dysentery antigen was injected for the first time had to be specially selected.

On primary injection, the antigen was excreted in the urine of all 8 dogs, although in two of them it was only found in traces, but in the remaining six in amounts which permitted calculations to be made.

The magnitude of clearance from dysentery antigens varied from 0.08 to 0.7 ml/min, which corresponds to 0.15 to 1.5% of the index of clearance of the simultaneously injected inulin or sodium thiosulfate (Table 1).

Repeated injections of dysentery antigen, which may be regarded as a process of immunization, were accompanied by increase in the indices of clearance of these antigens from the animal. This process is illustrated in Fig. 1, showing the results obtained in the dog Kashtanka.

The antigen clearance indices of the dogs subjected to primary and repeated injections but not immunized with vaccine were between 0.08-1.3 ml/min, which corresponded to 0.09-5.42% of the degree of filtration.

TABLE 1

Excretion of Dysentery Antigen by the Kidneys after Primary Injection

Name of dog	Clearance index of dysentery antigen (in ml/min)	Relation of degree of clearance of antigen to degree of filtration (in %)	Remarks
KashtanRa	0.7	1.54	Filtration estimated by thiosulfate
Khriza	Traces	—	"
Beliak	0.125	0.21	Filtration estimated by thiosulfate. Dog died after injection.
Laska	0.09	0.15	Filtration estimated by thiosulfate
El'ba	0.5	0.49	Filtration estimated by inulin
Seraia	0.08	0.40	" "
Ovcharka	0.1	0.23	" "
Martyshka	Traces	—	" "

Experimental Results of Administration of Dysentery Antigen to Dogs Previously Immunized with Dysentery Vaccine

Three dogs (Umnik, Laska and Lada) were immunized with alcoholized dysentery vaccine, obtained from the Moscow Institute of Epidemiology, Microbiology and Hygiene, according to the scheme: 0.5-1.0-3.0-5.0-5.0 ml at intervals of 5 days. Antigen was administered repeatedly to these dogs at intervals of 15 to 30 days.

The intensity of its excretion was such that the clearance index was from 0.20 to 2.43 ml/min, and in one case it amounted to 5.1 ml/min. Figure 2 shows the excretion of antigens from a dog after previous immunization with vaccine.

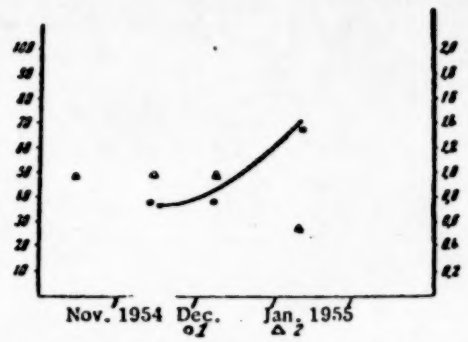

Fig. 1. Excretion of dysentery antigens after repeated injection into the dog Kashtanka.
1) Clearance index of antigen (on the right hand scale); 2) clearance index of thiosulfate (on the left hand scale).

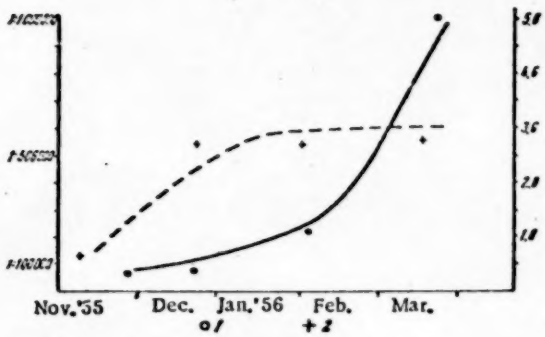

Fig. 2. Excretion of dysentery antigens on repeated injections into the immunized dog Umnik.
1) Antigen clearance index (on the right hand scale); 2) precipitin titer (on the left hand scale).

In order to solve the problem of specificity, we thought it desirable to set up an experiment in which a dog, immunized against dysentery, would be injected with another antigen, namely typhoid fever, in order to compare the degree of clearance of the latter with the degree of clearance of dysentery antigen injected in the same quantity.

The dog Lada, immunized with alcoholized dysentery vaccine, was given 2 injections of dysentery antigen, after which typhoid antigen was injected. Clearance of typhoid antigen was found to be 0.30 ml/min, amounting to 0.34% of the inulin filtration determined at the same time. Clearance of dysentery antigen amounted to 0.39 ml/min (0.53% of the filtration) if injected before the typhoid antigen and 0.85 ml/min (0.98% of the filtration) if injected afterwards.

Somewhat smaller values of clearance of typhoid antigens compared with clearance of dysentery antigens administered previously were obtained also in the dog Umnik which had been immunized to dysentery. Clearance of typhoid antigens in Umnik amounted to 1.62 ml/min (2.43% of the filtration), and of dysentery antigens injected subsequently, preceding the injection of typhoid – 1.8 ml/min (3.01% of the filtration).

Although these experiments do not permit the question of specificity of excretion of antigens from animals immunized to a given antigen to be finally answered, they do nevertheless suggest the existence of some degree of specificity in the excretion of dysentery antigens.

We also studied the problem of damage to the renal parenchyma. Control analyses of the urine before and after injection of dysentery antigen showed the appearance of traces of albumin in the urine after injection of antigen (transient proteinuria). The protein was estimated by the Robers-Stol'nikov method. In order to confirm the excretion of protein and to determine its nature we carried out anaphylaxis experiments with guinea pigs.

The plan and results of these experiments are shown in Table 2.

As seen in Table 2, in animals sensitized to urine taken from the dog after injection of antigen, and containing proteins, a strong anaphylactic shock was observed on injecting as an assaulting dose the serum proteins of a dog (with fatal results in 2 out of the four guinea pigs). In guinea pigs sensitized to urine taken from the dog before the injection of antigen and not containing proteins according to Heller's test, anaphylactic shock was also observed although to a weak degree. This fact suggests the presence even in normal urine of quantities of protein adequate for sensitizing a guinea pig, and in accordance with the findings of Rigas and Heller [10], who demonstrated by the methods of ultrafiltration and electrophoresis the presence of proteins in the healthy human urine.

Anaphylaxis experiments on guinea pigs, confirming the excretion of antigen in the urine, showed that at the same time there occurs excretion of serum protein. Such signs of damage to the kidneys as the presence of erythrocytes, hemoglobin, etc., were not found in our experiments.

TABLE 2

Schedule of Anaphylaxis Experiments on Guinea Pigs

Animal No.	Sensitization		Reaction in response to injection of an assaulting dose on May 17, 1956	Evaluation of severity of shock
	1st injection May 3, 1956	2nd injection May 5th, 1956		
1	2 γ of dysentery antigen	The same as in the 1st injection	Injection of 50 γ of dysentery antigen. Two min. later slight shivering and 8 minutes later, slight convulsions	+
2	1 ml of urine without protein (before injection of antigens)	Ditto	Injection of 1 ml of dog serum. One minute later severe convulsions in the lateral position	++
3	1 ml of urine containing proteins (after injection of antigen).	"	Injection of 1 ml of dog serum. One minute later severe convulsions in the lateral position. After 4 minutes, death.	++++
4	Ditto	"	Injection of 50 γ of dysentery antigen. After 7 minutes, periodic shivering, twitching.	+
5	"	"	Injection of 1 ml of dog serum. After 4 minutes strong convulsions, defecation and urination.	++
6	"	"	Injection of 1 ml of dog serum. One minute later convulsions in the lateral position, defecation and urination. Death after 6 minutes.	++++
7	Animal not sensitized		Injection of 1 ml of dog serum. No shock	−

It may be suggested that activation of the excretory function of the kidneys during excretion of antigens is accompanied by increased permeability of the glomerular membrane. This also shown by the appearance of a slight transient proteinuria. It must be pointed out that the fact of penetration of protein through the glomerular membrane is not regarded as a sign of renal damage and takes place in healthy animals (the so-called benign albuminuria) [5].

Phenomena similar to those which we observed, consisting of transient excretion of homologous serum protein have been noted in response to injection and excretion of hemoglobin and foreign protein ([7] and others).

From an assessment of the magnitude of the clearance indices of dysentery antigens obtained, and comparison with the degree of filtration as determined simultaneously in our experiments, we observe that percentage ratio between the clearance index of dysentery antigens and the total filtration is smaller than that calculated in the same way with dextran fractions with molecular weight of 28,500 (11% [11]). The clearance indices for dysentery antigens are closer to that of dextran with a molecular weight of 91,700 (1.2% of the total filtration), determined by Giebish et al., [6], and to the clearance of hemoglobin (according to Monke and Yuile) equal to 3% of the total filtration [9].

Regarding the mechanism of excretion of antigens it can be tentatively concluded that during excretion they are subjected to filtration and to reabsortive processes in the tubules. The difficulty in determining the renal threshold for antigens does not exclude tubular activity and it may depend, as with other proteins, on variations in the size of the molecules of different fractions of antigen.

Excretion of dysentery antigens is not accompanied in all animals by a uniform change in filtration. Increase of filtration, noted in certain animals, shows activation of filtration in the process of antigen excretion. However, such an increase in filtration does not always take place.

It is of interest that during excretion of phage, studied in L. A. Zil'ber's laboratory, uniform changes in filtration were also observed (V. A. Parnes et al., [3]). The absence of any parallel between filtration and antigen clearance shows that some process other than filtration is also concerned in the excretion of the antigen molecules. This process is the activity of the renal tubules. In this connection, dysentery antigens evidently are not distinguished from other macromolecular compounds undergoing filtration and reabsorption.

No strict parallel between antigen clearance and antibody titer in the blood could be observed in our experiments. The role of excretion of antigens by the kidneys was more pronounced in immunized animals, but no close relationship between the variations in antibody titer and antigen clearance index could be observed.

The increase in antigen clearance arising during immunization may depend on a lowering of the reabsorptive activity of the tubules and to an increase of filtration, brought about by immunization. This increase in the excretory function of the kidneys in respect to bacterial antigens arising during immunization may be regarded as a peculiar manifestation of the protective mechanisms, the pathophysiological mechanisms of immunity, not directly connected with antibodies or phagocytosis.

SUMMARY

In dogs dysentery antigens are excreted by the kidneys following their intravenous introduction. The indicator of clearance determined for dysentery antigens increases with their repeated introduction or after preliminary immunization by dysentery vaccine. Excretion of antigens is connected with a transitional proteinurea. However there are no signs of disturbed function of the kidneys. As to the mechanism of excretion of these macromolecules – one may assume that they are excreted by filtration in glomeruli with the following partial reabsorption in the tubules. It is probable that increased filtration of the antigens and decrease of their reabsorption takes place in immunization.

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THE INCUBATION PERIOD IN PASSIVE IMMUNITY TO TETANUS TOXIN

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From numerous experimental investigations and clinical observations we know that so-called passive immunity reaches its maximum degree of intensity not immediately after injection of immune serum but after some time. The existence of this characteristic incubation period became known soon after the discovery of the preventive properties of antitoxic serum [19], and different workers accounted for this phenomenon in different ways, depending on their ideas of the mode of action of antitoxin [2, 8, 21, 22]. The existence of this period in the development of passive immunity was regarded by some workers as an indication that passive immunity arises as a result of the action of antibodies on the body of the recipient. Despite the fact that it is now 70 years since this discovery was made, many aspects of the conditions of production of passive immunity and of the action of antitoxin in vivo have received insufficient study. Recently a number of works have appeared in which the significance of the action of tetanus antitoxin on the animal in creating specific resistance is once again examined [7, 11-14, 18].

Considering the practical and theoretical importance of these problems, we undertook the present investigation with the aim of explaining the features of development of passive immunity to tetanus depending on the conditions of administration of tetanus antiserum.

EXPERIMENTAL METHODS

The experiments were based on the production of experimental tetanus. This plan was selected not only because of the frequent interest in the study of passive immunity to tetanus but also because since the work of E. Bering and his co-workers [2] experimental tetanus has become one of the main experimental devices for solving a number of general immunological problems. This has not lost its importance even today [16].

In the experiments we used tetanus antiserum purified and concentrated by the method Diaferm-3 IEM (series 1881), and dry tetanus toxin (series No. 580 IEM AMN SSSR). This latter was first dissolved in different volumes of physiological saline containing glycerin (pH = 6.9). The required dilutions of toxin were made just before each experiment. The investigations were carried out on white rats weighing 125-140 g. The severity of the disease was rated by mortality, survival time of animals who later died, and clinical picture. In order to describe the signs and symptoms of local tetanus we used special symbols; the degree of resistance of the animals to the toxin was judged by the clinical course of the disease. The resistance was conventionally expressed in points (Table 1).

A lethal dose of toxin was chosen as the dose which on injection subcutaneously in a volume of 0.2 ml in the outer surface of the left thigh, caused death of all the animals on the 4th and 5th day. In the first 3 series of experiments tetanus antiserum was given in a dose of 5 units; this dose corresponds roughly to that used in practice for prophylaxis of tetanus (calculated per 1 kg body weight).

TABLE 1

Symbols Used in Describing the Clinical Course of Local Tetanus and the Degree of Resistance of the Animals

Clinical picture	Symbols	Degree of resistance in points
Absence of any signs of disease.	−	10
Barely perceptible increase of extensor tonus (more readily observed rats are lifted up by their tails).	±	8
Clearly expressed increase of extensor tonus. This is mainly observed when the animal moves about.	±	6
Considerable increase of extensor tonus. The paw is semiflexed, the springing joint does not lie on the surface of the ground.	+	4
Sharp increase of extensor tonus. The paw is drawn backwards, and on walking only the phalanges touch the ground, and movement in the joints, especially the springing joint, is restricted.	++	2
Total local tetanus. The paw is extended backwards and takes part in no movements; muscular rigidity; the sole and phalanges are extended; movement in the springing joint is absent, while occasionally a trace of movement may be preserved in the other joints.	+++	0

Estimation of antitoxin in the blood of the rats was made by the usual method of titration in mice. In control tests normal rat serum was used.

The experimental results were treated by statistical variance analysis.

EXPERIMENTAL RESULTS

The purpose of the first series of experiments was to discover how quickly the maximum degree of resistance of animals arises after intramuscular injection of tetanus antiserum.

In these experiments tetanus antiserum was injected (5 units in a volume of 0.2 ml) into the posterior group of muscles of the right thigh, and tetanus toxin (1 lethal dose) − into the posterior group of muscles of the left thigh.

As seen from Table 2 after injection of serum immediately before injection of toxin, none of the animals showed any protection against the disease; in all the rats total local tetanus developed, with characteristic muscular rigidity and restriction of movements at the joints. Three hours after injection of the serum the resistance of the animals was increased (local tetanus did not reach its maximum development), after 6 hours further increase could be observed, and after 12 hours the resistance of the animals was at its highest. Differences in the degree of resistance of the animals of each successive group compared with the resistance of the animals of the preceding groups were significant in every case. The resistance of the animals to tetanus 24 hours after injection of serum was practically the same as that after 12 hours (variations in the degree of resistance are statistically not significant).

Thus by intramuscular injection of tetanus antiserum and tetanus toxin in different places, passive immunity reaches its maximum development not at once after injection of the serum, but after a definite period of time. This result is in accordance with the reports in the literature on the subject.

The next series of experiments were intended to explain the rate of accumulation of antitoxin in the blood of the animals under the conditions of the experiment.

The rats were exsanguinated by section of the cervical vessels. From the blood, serum was prepared

and tested for its antitoxic properties on the day after it was drawn. Each test was carried out with a mixture of equal quantities of sera from 4 rats (0.4 ml of each serum). The entire experiment was repeated 3 times. In the whole experiment 60 rats were used.

As seen from Table 3, as the interval after injection of serum intramuscularly increases, so also does the amount of antitoxin in the blood, and it reaches its maximum in 12 to 24 hours (variations in the antitoxin content after 3, 6 and 12 hours from the injection of serum are statistically significant). After 24 hours the content of antitoxin in the blood was roughly the same as after 12 hours.

TABLE 2

Prophylactic Effect of Tetanus Antiserum Injected at Various Intervals Before Injection of Toxin

Time between injections of serum and toxin (in hours)	Number of animals in the experiment	Clinical course of the local tetanus	Resistance in points (M ± m)
0	10	+++	0
3	15	++	2±0.52
6	15	<+	4.6±0.69
12	10	<÷	9±0.3
24	10	<÷	9.2±0.41

Note: Intermediate degrees of development of the disease are signified by the additional signs > or <.

The results obtained agree with those of other investigators [20, 21]. Any differences consist in the fact that in the opinion of other workers cited, the maximum level of the antitoxin content of the blood may be observed in some cases on the 2nd-3rd day after subcutaneous or intramuscular injection of tetanus antiserum. This difference may be explained by the fact that we used purified serum, the size of the immune globulin of which reduced by enzyme digestion at the time of purification [1]. Thanks to this purification by the Diaferm-3 IEM method the serum is more rapidly absorbed than the native serum [1].

Comparing the results of the two series of experiments a parallel may be observed between the change in the level of the blood antitoxin and the degree of resistance of the animals. As can be seen from the figure, the curve of growth of resistance of the animals corresponds to the curve of growth of antitoxic properties of the blood.

The results obtained enable the conclusion to be drawn that the degree of resistance of the animals to tetanus toxin in passive immunity is determined by the level of the blood antitoxin content, derived from the site of injection. This conclusion was confirmed by the results of an experiment in which tetanus antiserum was injected in the same dose (5 units) directly into the blood stream (into the vein of the right calf). In these conditions, the simultaneous injection of toxin into the limb muscles produced in the majority of animals merely an insignificant rise in the extensor tonus (from − to ±; it reached ± in only 1 rats out of 20). At the same time, all of 20 rats receiving serum into the muscles of the right thigh developed total local tetanus (+++) with characteristic extensor rigidity and restricted movement in the joints.

Thus on injecting tetanus antiserum directly into the blood stream with the object of creating the required concentration of antitoxin in the minimum time, we observed the same degree of resistance in the animals which develops roughly 9 to 12 hours after intramuscular injection of the same dose of serum.

TABLE 3

Content of Antitoxin in the Blood of Rats at Various Intervals After Injection of Tetanus Antiserum into the Limb Muscles

Interval between taking blood and injection of tetanus antiserum (in hours)	Series of experiments			
	I	II	III	mean
	content of antitoxin in units			
0	0	0	0	0
3	0.09	0.09	>0.08	0.09
6	<0.14	0.13	0.15	0.14
12	0.18	0.19	0.17	0.18
24	>0.2	<0.18	0.17	0.18

TABLE 4

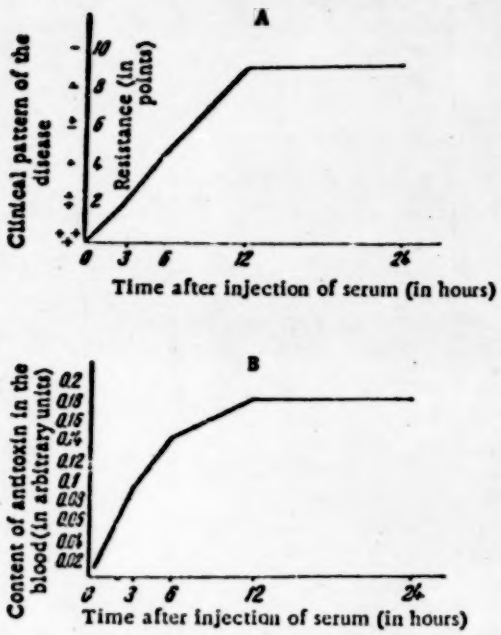
Prophylactic Effect of Tetanus Antiserum, Injected into the Blood as a Mixture with Toxin or Separately at Various Intervals Before Intravenous Injection of the Toxin

Group No.	Conditions for injection of serum	Dose of serum – 0.02 units				Dose of serum – 0.01 units			
		number of animals in experiment	number developing the disease	number which died	mean survival period, days	number of animals in experiment	number developing the disease	number which died	mean survival period, days
1	As a mixture with toxin	5	0	0	—	5	1	0	—
2	Just before injection of toxin (into another vein)	5	0	0	—	5	4	0	—
3	3 hours before injection of toxin	5	0	0	—	5	5	1	9
4	6 hours before injection of toxin	5	0	0	—	5	5	4*	5,8
5	12 hours before injection of toxin	5	4	0	—	5	5	5	3
6	24 hours before injection of toxin	5	5	1	6	5	5	5	2,5
7	Toxin control (serum not injected	5	5	5	1,2	5	5	5	1,2

* One rat died with the appearance of exhaustion.

The next series of experiments were for the purpose of discovering the form of the passive immunity appearing in response to injection of both toxin and serum into the blood stream.

Doses of 0.02 and 0.01 units of serum in a volume of 0.2 ml were injected into the vein of the right calf; toxin (3.5 lethal doses in a volume of 0.15 ml) was injected into the vein of the left calf at different intervals after injection of the serum. In addition a portion of the animals received a mixture of toxin and serum which had been prepared just before injection into the vein (not kept in the incubator). The results of the experiments are shown in Table 4.



Change in the resistance (A) and blood antitoxin content (B) of rats at various intervals after intramuscular injection of tetanus antiserum.

As seen from Table 4, in this experiment the best prophylactic effect was observed in those cases where the serum was injected either as a mixture with toxin or simultaneously with it. As the length of time between injection of serum and injection of toxin increased, so the prophylactic effect of the tetanus antiserum decreased, and reached its minimum after 12 to 24 hours.

The results of this experiment are thus diametrically opposed to those which were obtained in the experiment in which toxin and serum were injected into the muscles of different limbs. In this present experiment no incubation period could be observed in the development of passive immunity after injection of tetanus antiserum.

The results obtained suggest that the incubation period in passive immunity is the period of resorption of antitoxin and the time for its distribution throughout the body, thereby creating optimal conditions for fixation of the toxin at the site of its injection (or formation) and of its action. Of great importance in this respect is a necessary concentration of antitoxin in the blood, permitting not only blockage of the humoral route of spread of toxin but also access of antitoxin to the appropriate sections of the nervous system on which the toxin acts. The possibility of access of tetanus antitoxin from the blood to the central nervous system may now be considered to be proved [3, 4, 9, 10]. For this reason the duration, presence or absence of the time required to produce the maximum prophylactic effect of antitoxic serum will be determined not only by amount and site of injection but also by conditions of injection and spread of the toxin.

These facts which have been presented suggest that the prophylactic action of tetanus antitoxin is connected primarily with its power to fix toxin. If the prophylactic effect of tetanus antiserum were due to its action on the macroorganism, the existence of an incubation period would be observed in all cases of passive immunity, just as occurs in any reaction of the body to any stimulus. This naturally does not dismiss the importance of the action of the serum proteins, including immune protein, on the body as an additional stimulus modifying the reactive basis of the animal. The importance of this mechanism, like the importance of changes in reactivity due to other stimuli, may vary in different conditions.

From the foregoing it will be understood why in the usual conditions of passive immunity (in the absence of special stimuli modifying the reactivity of the body) a direct relationship is particularly clearly observed between the degree of resistance of animals and their blood antitoxin level. As may be seen from the results presented, even comparatively fine differences in the clinical pattern of local tetanus correspond to changes in the content of antitoxin in the blood. In active immunity such a relationship may not always be present or so clearly expressed [5, 6, 15].

SUMMARY

It was established in experiments on white rats that the time of the maximal immunity against tetanus depends on the method of introduction of antitetanus serum and tetanus toxin. The resistance of the animals to the toxin depends directly on the content of the antitoxin in their blood. The mechanism of action of tetanus antitoxin and conditions of appearance of the passive immunity are discussed.

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ONCOLOGY

THE EFFECT OF EXTRACTS OF HIPPOPHAE RHAMNOIDES UPON THE DEVELOPMENT OF TUMOR TRANSPLANTS IN ANIMALS

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A previous communication [1] has already reported the fact that an alcohol extract of the bark of Hippophae rhamnoides (sea buckthorn) inhibits the growth of an Ehrlich tumor introduced subcutaneously. The present study presents the results of a further investigation of the antitumor properties of this extract.

EXPERIMENTAL METHODS

The extract was prepared by M. F. Petrova in the laboratory of the chemistry of native extracts (Chief – G. P. Menshikov). The specimens of the plant had been collected in the summers of 1955 and 1956 by the Central Asiatic expedition accompanied by P. S. Massagetov. The method for obtaining the alcoholic extracts has been described previously [1]. After the removal of the alcohol, the extract was treated with gelatin or talc for the purpose of separating from it the woody, bark substances.

The doses were calculated in mg of the dried extract per kg weight of the animals.

The treatment of the animals with transplanted tumors was begun at various time intervals after the inoculation, the actual time depending on the initial growth of the particular tumor strain. The injections were given daily. The treatments were continued from 10 to 20 days, this factor also being varied in dependence on the individual characteristics of the growing tumors.

In order to arrive at an opinion as to the effectiveness of the preparation against the growing tumors, the average weights of the tumors in the control group would be compared with the average weight found in the experimental. This difference was then expressed as per cent of the average in relation to the weight of the controls and served as the basic indicator of the inhibiting effect of the preparation upon the tumor growths. In addition, we measured the growth of the tumors in the experimental and control animals by taking the comparative diameters of the tumor swellings every 5 days.

EXPERIMENTAL RESULTS

Experiments on mice and rats [1] had already shown that the extract was much more toxic when gelatin had been used to remove the tannates than when talc had been employed for that purpose; there was but little difference in toxicity between the gelatin treated substance and the native material still containing the tannates so that subcutaneous or intra-abdominal injections caused marked irritation and could be used only in small doses. In the majority of the experiments we used the extract which had been treated with the talc, as this preparation did not have a local irritating action.

The lethal dose (LD_{100}) of the extract purified with the talc, by adding talc, when injected intraabdominally

into rats, was 1 g of the dry substance per 1 kg weight of the animals; deaths beginning 3-4 hours after the injection. The average lethal dose (LD_{100}) was 750 mg/kg, while the maximum bearable dose was 600 mg/kg.

In the rats which died (LD_{100}) there was noted cyanosis of the extremities, ears and nose. At autopsy there was generalized venous hyperemia of the abdominal organs as well as hyperemia and hemorrhages into the lungs in addition to thymic edema.

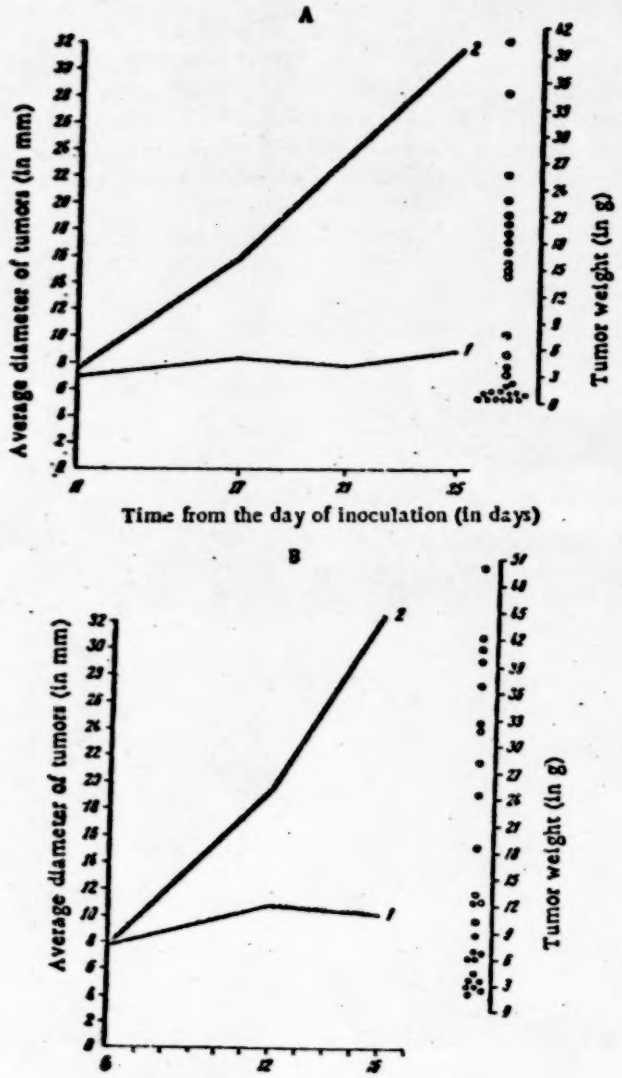
In subsequent experiments we established that repeated injections into the rats of 400 mg/kg doses neither caused death nor produced marked alterations in the organs. However, after each injection of the extract there could be observed inhibition and weakening of the motor activity of the animals followed by ataxia and simultaneous increase in the respiratory rate while the respiratory movements became more shallow. With a dose of 600 mg/kg the respiratory repression continued for several hours.

In rabbits we also observed the effects of extract injections upon the blood picture. For 9 days 3 rabbits received daily subcutaneous injections of 150 mg/kg of the extract cleared with the talc; daily leucocyte counts were made and an erythrocyte count was made initially and at the end of the course. The extract failed to produce any quantitative changes.

We studied the action of the alcohol extract of the bark of Hippophae rhamnoides upon 10 different transmittable rat and mouse tumors: small doses of the native extract, small doses of the gelatin-cleared extract and, finally, various doses of the extract cleared with the talc (see Table).

The Effects Produced by Extracts of the Sea Buckthorn Bark Upon Tumor Growth

Extract	Dose (in mg/kg)	Method of introduction	Tumor	No. of animals		Days after inoculation: treatment with extract begun	Length of treatment (days)	No. of days until death		Inhibition of tumor growth in expt'l. animals, (% of controls)
				experimental	control			experimental	control	
Native	150	Subcutaneous	Ehrlich mouse tumor	15	15	5	10	0	2	51
	150	»	Mouse hepatoma	20	20	6	14	1	1	50
Cleared with gelatin	100	Subcutaneous	Ehrlich mouse tumor	20	20	6	10	3	1	42
	150	»	Rat carcinoma of Guerin	14	14	12	11	0	2	54
	150	»	Rat sarcoma 45	15	15	1	14	1	2	29
Cleared with talc	200	Subcutaneous	Ehrlich mouse tumor	20	20	5	12	0	0	61
	400	Intra-abdominally	»	15	15	5	11	4	6	69
	200	Subcutaneous	Mouse mammary cancer	15	15	11	18	0	1	64
	400	»	Mouse cancer of the forestomach	17	17	5	18	2	0	19
	400	Intra-abdominally	Mouse melanoma	21	21	20	21	5	3	60
	150	Subcutaneous	Crocker mouse sarcoma	25	25	6	10	1	2	44
	400	»	M-1 rat sarcoma	16	16	6	11	3	3	82
	400	Intra-abdominally	Rat sarcoma 45	15	15	7	15	0	0	93
	200	Subcutaneous	Walker rat carcinosarcoma	15	15	4	10	1	0	70
	400	»	Rat carcinoma of Guerin	15	15	11	14	1	0	96


A
Average diameter of tumors (in mm)
Tumor weight (in g)
Time from the day of inoculation (in days)
B
Average diameter of tumors (in mm)
Tumor weight (in g)

Effect of sea buckthorn extract upon the growth of Guerin carcinoma (A) and M-1 sarcoma (B) (experiments No. 15 and No. 12).
1) Experimental; 2) control. Circles indicate distribution of tumors by weight at the end of the experiment. • – experimental, o – control.

As can be seen from the Table, all three extracts retarded the growth of a subcutaneously inoculated Ehrlich tumor to about the same extent, i.e., 50-60%.

The native extract was tested on mice inoculated with mouse hepatoma C_3HA (strain obtained by V. I. Gel'shtein with the aid of orthoamidoazotoluol) and it was noted that it produced tumor inhibition.

The extract, cleared with gelatin had a feeble antitumor effect upon rat sarcoma 45 and a more pronounced effect upon Guerin carcinoma whose original strain had been obtained from a spontaneous rat uterine carcinoma.

The extract cleared with talc and used in doses of 400 mg/kg inhibited markedly the growth of Guerin carcinoma, sarcoma 45 and sarcoma M-1 (see Figure) but complete resolution of the tumors did not occur; in some nodes weighing several score mg the microscope would show individual, undamaged blastomatic cells.

A marked effect was observed with experiments on the Walker carcinosarcoma 256 (strain obtained from a spontaneous carcinoma of rat mammary gland), in experiments with mammary carcinoma (RMZh) in hybrid C_{57} x A (strain obtained by L. A. Bolonin from spontaneous mouse mammary carcinoma), in experiments with melanoma of Harding–Passy on mongrel mice, however, the tumors did not dissolve completely. The extract was particularly resisted by strain PRZh (gastric cancer)in mice breed CC_{57} (strain obtained by Dobryninin by the action of 9 : 10 dimethyl-1,2-benzanthracene).

The results of these experiments has been to demonstrate that the bark of Hippophae rhamnoides has a principle which can be extracted with alcohol and which will inhibit markedly the growth of 8 strains of tumors out of the 10 tested; in other words, this principle has a broad antitumor spectrum of activity. However, the toxicity of the extract is such that it cannot be offered, as yet, for clinical testing; further study of the essential active substances must be continued.

SUMMARY

The antitumor activity of alcohol extracts of the bark of Hippophae rhamnoides as well as the bark of the tree itself was tested upon 10 different forms of transplantable tumors found in mice and rats.

Tumors were markedly inhibited in 8 of the 10 types of tumor tested even if complete resorption failed to be observed.

The preparation is still very crude and toxic. More studies of the active ingredients are needed before the material will be suitable for clinical trial.

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EXPERIMENTAL BIOLOGY

THE PRESENCE AND DISTRIBUTION OF ALKALINE PHOSPHATASE IN THE ORGAN OF CORTI IN ANIMALS IN A STATE OF RELATIVE REST AND DURING AUDITORY STIMULATION

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The present communication presents the results of histochemical examination of the organ of Corti for alkaline phosphatase by the Gomori method [4].

Thirty organs of Corti from 30 animals including 18 cats (10 kittens and 8 adult), 6 rabbits and 6 guinea pigs, were examined in whole-area preparations.

In the first, control, series of experiments the 10 animals concerned were maintained in a state of relative rest prior to decapitation. In the second series of experiments the same number of animals was subjected to auditory stimulation by sounds of high frequency – 1500 cps (95 decibel)–for one hour prior to decapitation. In the third series a similar number of animals was subjected to auditory stimulation by low frequency sounds – 300 cps (95 decibel) – for one hour prior to decapitation.

Whole-area preparations of the organ of Corti in the case of control animals show a clear picture which indicates, at first glance, diffuse activity of the enzyme which becomes gradually attenuated in the middle and particularly the inferior convolutions. A concentration gradient is thus observed which is spirally arranged with respect to intensity being most marked in the superior convolution and declining towards the inferior one. There is no doubt that the presence of such a gradient is connected with functional differences between the elements of the organ of Corti dependent on their situation on one or other level of the cochlea.

The Gomori stain gives rise to the greyish-brown coloration in all the structural elements of the organ of Corti which resembles closely the iron hemotoxylin stain. The intensity of color depends on the length of incubation: prolonged incubation (18 hours) is associated with deep coloration of the preparation, shorter incubation (6 hours) with a lighter stain. At high magnification it becomes apparent that despite the generalized staining of the preparation individual structural elements of the organ of Corti are markedly distinct, in other words the concentration of alkaline phosphatase in various structural elements of this organ is far from identical, indicating differences in the enzyme's activity in various cellular structures of the organ.

Alkaline phosphatase is well demonstrated in the epithelium of Reissner's membrane facing the endolymph cavity of the organ of Corti. Alkaline phosphatase activity is clearly seen at intercellular borders which have the appearance of fine, regular dark brown lines delineating polygonal epithelial cells. These borders are considerably more definite than those seen in preparations stained by silver nitrate impregnation. The cytoplasm of the epithelial cells is vacuolated, clear and contains practically no enzyme although isolated granules with high content of the enzyme are at times encountered. The nucleus is distinguished by its large size with clearly defined nuclear membrane. The karyoplasm consists of more or less regular granules with considerable concentration of the enzyme. Against their background one or more nucleoli stand out with high enzyme concentration. The enzyme is absent from the connective tissue part of the membrane.

It is also absent from the endothelium facing the perilymph aspect of the scala vestibuli. The endothelial nuclei contain the enzyme.

The basilar membrane is distinguished by alkaline phosophatase activity at the intercellular borders of the endothelium facing the perilymph of the scala tympani. The enzyme is also present in the cytoplasm of the endothelial cells and especially in the nuclei. There is undoubted enzyme activity in the connective tissue radial fibers of the basilar membrane. These are uniformly stained throughout their length to a brown color. Alkaline phosphatase is absent from the basal substance of the basilar membrane.

Fig. 1. Alkaline phosphatase in the outer hair cells of the organ of Corti at the level of the middle (2nd) convolution of a young rabbit in a state of relative rest. Whole-area preparation. Average magnification (oc. 7x, obj. 40). Microphotograph.

No alkaline phosphatase activity can be demonstrated in the tectorial membrane of the organ of Corti. It remains absolutely clear irrespective of the length of incubation. The enzyme is also completely absent from the Kolliker membrane. Low concentrations of the enzyme are seen in the inner hair cell cytoplasm; higher concentrations are present in the nucleus, the nuclear membrane and particularly in the nucleolus. The enzyme concentration is high in the sensory hairs of the inner hair cells. It must be pointed out that there is slight diffuse staining of the phalanges and phalangeal cells which indicates the presence of alkaline phosphatase.

The outer hair cells stand out prominently owing to enormous concentration of the enzyme. They are stained deep brown and together form 3 dark strips, divided by light interlayers of the phalanges, which extend along the whole organ of Corti on all the convolutions of the cochlea (Fig. 1). However, the concentration of alkaline phosphatase in the hair cells follows a definite downward trend along the spiral from the upper to the lower convolutions (spiral downward gradient). At high magnification each hair cell can be studied individually. The hair cell is distinguished by dark, faintly granular cytoplasm with uniformly distributed concentration of the enzyme. The nucleus is sharply defined by the nuclear membrane. The karyoplasm is granular and contains one or several nucleoli with marked enzyme activity. The apical surface of the hair cells shows the sensory hairs very clearly owing to high concentration of the enzyme. The hairs of each cell form together a characteristic pattern in the form of the letter V with its apex pointing towards the Hensen cells (Fig. 2). In the case of the outer hair cells, however, the concentration of the enzyme increases on approaching the Hensen cells; the enzyme concentration is higher in the second layer than in the first and is still higher in the third one (see Fig. 1). There is thus a radial gradient of phosphatase content in the outer hair cells. The content and distribution of glycogen coincide remarkably with the content and distribution of alkaline phosphatase in these cells.

Low concentrations of the enzyme are found in the pillar cells where the inner hair cells are separated from the outer hair cells by a light, regular strip corresponding to the tunnel. The alkaline phosphatase activity is also slight in the cells of Deiters. It is considerably higher in the cells of Hensen and Claudius. Enzyme activity is demonstrable in the region of the intercellular borders and in the granular cytoplasm of the Hensen cells and also the cells of Claudius. The cell nuclei are sharply defined by the nuclear membrane and show granular karyoplasm and intensely stained nucleoli.

The vascular strip shows extraordinarily high alkaline phosphatase activity, particularly in the vascular endothelium. The borders between the epithelial cells, however, are not discernible, their cytoplasm and nuclei being deeply stained even on short incubation.

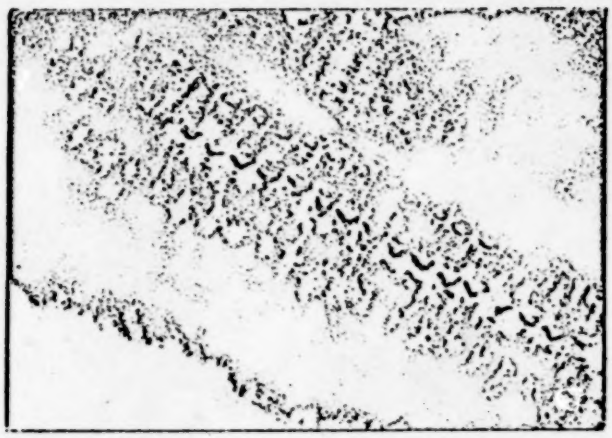

Fig. 2. Alkaline phosphatase in the hairs of the outer hair cells of the organ of Corti at the level of the inferior (3rd) convolution in a young cat. Whole-area preparation. Immersion magnification. Microphotograph.

The enzyme is found, in low concentration, in many ganglion cells of the spiral ganglion. Its activity is slight both in the cytoplasm and in the nucleus, although it is somewhat higher in the cytoplasm of the nerve cells than in the nucleus. The latter shows greater activity in the nucleolus. No neurofibrils are seen. Considerably higher enzyme activity is noted in the cytoplasm and nuclei of the satellites, the connective tissue and the endothelium of the surrounding blood vessels.

No alkaline phosphatase is, as a rule, found in the nerve fibers situated in the vicinity of the ganglion cells. It is, however, often found in the dendrites which pass to the basilar membrane and end on the hair cells. The individual nerve fibers here are seen as fine, winding threads forming T-shaped or knee-shaped ramifications; such fibers are seen both in the region of the tunnel and under the hair cells at the base of which they may end in pinhead-like enlargements. Such is the picture of alkaline phosphatase distribution in the organ of Corti of control animals.

The differences between our data concerning the alkaline phosphatase picture in the organ of Corti in cats, rabbits and guinea pigs and data obtained for bats [5] should, evidently, be explained by the difficulties encountered in the preparation of paraffin sections from the inner ear of bats. The advantage of the whole-area preparation method is quite obvious.

In the second series of experiments – following auditory stimulation by sounds of high frequency – the character and distribution of alkaline phosphatase in the organ of Corti underwent a change. The concentration of the enzyme diminished to some extent in Reissner's membrane, at the intercellular borders and in the cytoplasm as well as the nuclei of the epithelium. The connective tissue radial fibers of the basilar membrane in the region of the third and part of the second convolution show marked decrease of enzyme concentration with complete disappearance of the enzyme at the end of the third convolution. This was associated

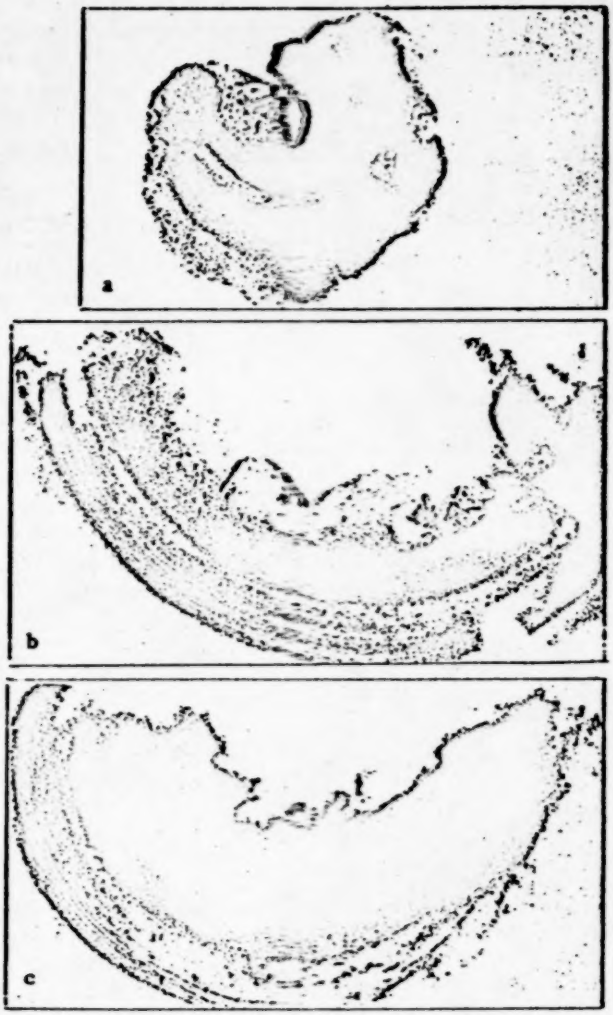

Fig. 3. Gradient of diminution of alkaline phosphatase concentration in the organ of Corti of young cat: a) superior (1st) convolution; b) middle (2nd); c) inferior (3rd).
Whole-area preparations. Magnifying lens, low magnification.

with a general drop in the concentration of alkaline phosphatase in the structural elements of Corti's organ situated on the basilar membrane (Fig. 3, a, b, c). In other words, the concentration of the enzyme did not differ from the control in the region of the superior convolution (Fig. 3 a) but to some extent in the middle convolution (Fig. 3, b) and particularly in the inferior convolution (Fig. 3, c) all the structural elements of the organ of Corti, including the hair cells, showed general diffuse lowering of alkaline phosphatase concentration which, in the case of the most inferior region amounted to practical disappearance.

Such a general fall in alkaline phosphatase activity coincides with a number of features characteristic of the excited state of the hair cells in the inferior and to some extent the middle convolutions. The inner hair cells usually show little change apart from some general decrease of coloration. The outer hair cells not

only show progressive decrease of coloration in ascending the spiral of the cohclea but also acquisition of a rounded shape as the result of contraction; this rounded shape is characteristic of the state of excitation. The cell cytoplasm is vacuolated. Most of the nuclei are, as a rule, enlarged considerably while some tend to shrink. The karyoplasm of the enlarged nuclei is lighter than in the control and this is also true of the nuclear membrane. The lighter nucleoli are displaced towards the periphery of the nucleus to lie adjacent to its membrane. The shrunken nuclei are more deeply staining. Their structure cannot be discerned. The hairs situated at the apical surface of the cells also show a gradual fall in enzyme concentration. The angle formed by them becomes obtuse instead of acute. It must be mentioned that the concentration of enzyme is always somewhat higher in the hair cells than in the surrounding phalanges (Fig. 3,b,c). In those cases where the enzyme is present it is found in larger quantities in the outer layer of the hair cells (radial gradient).

Consistent decline in enzyme concentration is also observed in the pillar cells and the cells of Deiters, Hensen and Claudius. A similar fall in concentration is noted in the elements of the vascular strip especially in the region of the third convolution. The concentration of the enzyme is markedly lower in the ganglion cells of the spiral ganglion, in the satellites and in the nerve fibers passing to the basilar membrane.

In the third series of experiments, in which auditory stimuli of low frequency were used, analogous changes were seen to occur. But, unlike the experiments in the second series, these changes were confined to the superior and part of the middle convolutions of the cochlea. The concentration of alkaline phosphatase was thus lower than control only in the lower convolutions of the cochlea in the second series of experiments and in the superior convolution in the third series. The middle convolution occupies an intermediate position. Consequently changes in enzyme concentration in the organ of Corti are related to the effect of sound stimuli of different frequencies but the general gradient relationships established in control experiments are maintained.

It seems to us that this cannot be unrelated to the similar gradient for glycogen which has been found by us in the organ of Corti [1]. The concentration of glycogen in the hair cells is also connected with the functional changes in the organ. This coincidence no doubt indicates that alkaline phosphatase participates in the processes of breakdown and synthesis of phosphate esters, i.e., in carbohydrate metabolism [4, 6]. There is suggestive evidence that changes in the alkaline phosphatase concentration in the cytoplasm and particularly in the nucleus presumably indicate a connection between this enzyme and nuclein metabolism [3, 5]. The fact that the fall of enzyme concentration does not constitute a fall of its enzyme activity in the true sense of the word, as shown by the experiments described, is of great interest. Quite to the contrary, enzyme concentration declines at the very areas of the organ which are functioning at the given moment with the greatest intensity and utilize the maximal amount of alkaline phosphatase. The lowest concentration of the enzyme is thus found to be connected with its greatest activity i.e., with its maximal participation in catalytic reactions. Such a histochemical interpretation of the "activity" of the enzyme (alkaline phosphatase) would seem to find support in similar data obtained on Vater-Paccini corpuscles [1].

SUMMARY

The distribution gradient of alkaline phosphatase was established as a result of histochemical investigation of the organ of Corti.

The character and the distribution of alkaline phosphatase in the organ of Corti changed after stimulation by high frequency sound waves. The concentration of the enzyme in the superior convolution did not differ from that in the control group. There was a decrease of the enzyme in the inferior convolution and its complete absence in the lowest portion. The medial convolution occupied an intermediate position.

Analogous changes were observed when the stimulation was accomplished by sound of low frequency.

The concentration of acid phosphatase in the organ of Corti underwent a change depending on the difference in frequency of the sound used for stimulation. However, the gradient ratio which was established in control animals remained unaltered.

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THE PROBLEM OF SPECIALIZED MUSCLE FIBERS IN MAMMALS

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The theory that there is present in mammals specialized muscle fibers adapted to tonic or tetanic activity can be debated. Thus, S. N. Gorshkov and E. A. Guseva [1], having obtained both tonic and tetanic contractions in the same mammalian muscles subjected to various stimulating conditions, are inclined to the opinion that there is but one single substrate effecting both tonic and tetanic reactions. S. Kuffler [6] holds to the same opinion, asserting that such specialized muscular fibers as are present in frogs are absent in mammals. However, R. R. Sharipova [5], on the basis of her investigations, arrived at the conclusion that specialized muscular mechanisms are present in mammals. E. K. Zhukov [4] also considers the presence of specialized muscular mechanisms in mammals to be a reality.

Evidently, some light could be shed on this matter by carrying out experiments on individual fibers separated from mammalian muscles. In the literature available to us we did not find any investigations carrried out on isolated mammalian muscle fibers with the aim of discovering fibers specialized for tetanic or tonic function. In the work of Honcke [7], where investigations on the structure and function of isolated live mammal striated muscle fibers are described, there are no experiments devoted to revealing specialized tonic and tetanic fibers. We therefore considered it expedient to undertake an investigation of this nature.

EXPERIMENTAL METHODS

The methods of preparing, sustaining, and recording the contractions of the muscle fibers do not differ from those adopted by E. K. Zhukov and L. I. Leushina [2, 3] in experiments with isolated frog muscle fibers.

Initially experiments were carried out on fibers extracted from the heel muscle of a rat (that known in the literature as the red static muscle). The fibers were extracted both from the surface area of the muscle close to the nerve junction and from other parts of the muscle. The second part of the experiment was carried out with fibers separated from the sole muscle. The preparation of the fibers was undertaken on a heated stage at a temperature of 30-32° under a binocular microscope. The preparation technique here is somewhat more complicated than that for preparation of frog muscle fibers since the connective tissue membrane of the rat muscle is thicker and the muscle fibers themselves are finer and looser than in the frog. After separation the fiber was placed in Ringer-Locke solution warmed to 30-32°, and left there for some time in order to better ascertain whether it had suffered damage during preparation. After examination under the microscope the fiber, ready for experimentation, was placed in a parallel-sided glass chamber and supported by a thread fastened to the tendon on the anode of nonpolarized Dubois-Raymond electrodes. To the other tendon a tension lever was attached. The cathode of the nonpolarized electrodes consisted of a tuft of silk thread immersed in the solution within the chamber.

The constancy of the temperature within the chamber was ensured by a special heater. The temperature fluctuation did not exceed 0.5°. Thus, from the beginning of preparation to the end of the experiment the muscle fiber was under a temperature equal to that within the rat's body.

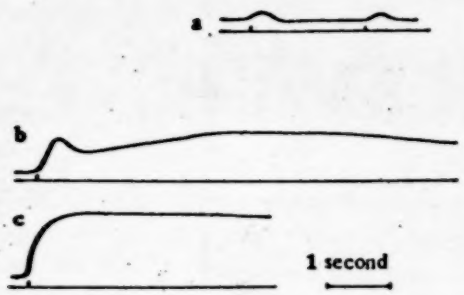

Fig. 1. Contractions in response to single stimuli of fibers of the transitional type extracted from the heel muscle of a rat.
a) With a distance between inductor coils of 15 cm; b) with 10 cm; c) with 5 cm.

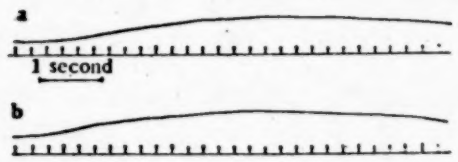

Fig. 2. Contractions in response to stimulation by an induction current for a distance between inductor coils of 8 cm displayed by fibers of the tonic type extracted from the heel muscle.
a) At the beginning of the experiment; b) after 1 hour and 15 minutes.

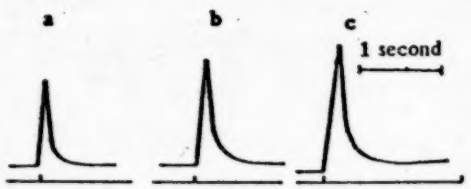

Fig. 3. Contractions in response to single stimuli of a group of 3 fibers of the tetanic type extracted from the sole muscle.
a) With a distance between inductor coils of 15 cm; b) with 10 cm; c) with 5 cm.

Stimulation of the fiber was by single induction pulses from an induction coil with a primary circuit tension of 6 volts. The technique for recording fiber contractions did not differ from that described in the work of E. K. Zhukov and L. I. Leushina.

EXPERIMENTAL RESULTS

The contraction of heel muscle fibers separated from the surface of the muscle, when subjected to near-threshold stimulation, is characterized by a fairly smooth increase and a slow decrease in the contraction curve. On a myogram these contractions have a cupola shaped appearance (Fig. 1,a). The contraction of fibers obtained from deep within the heel muscle takes place much faster. Their amplitude is greater and on a myogram they have the appearance of peaks with sharp apices. With stronger stimulation of these fibers, as a rule, a tonic component appears with the shape of a more or less pronounced slow supplementary contraction wave (see Fig. 1,b). The stronger the stimulation, the more pronounced is the tonic part of the contraction and the quicker it develops. With sufficiently strong stimulation one may observe that the tetanic part of the contraction and the tonic component merge together, as it were. One obtains a generally high contraction which quickly develops and slowly decreases after the excitatory stimulus (see Fig. 1, c). Evidently, from the nature of its contraction a fiber of this type can be considered a transitional fiber displaying both tonic and tetanic contraction components [3].

This is the means by which the overwhelming majority of the fibers contract. This brings to mind the great diversity of the transitional fibers within which fast and slow contraction components are combined in the most variable proportions.

Aside from these, fibers may be extracted from the heel muscle which contract only on stimulation of considerable strength (7-5 cm on the induction coil). The character of their contraction is extremely similar to the contraction of the tonic fibers of the frog (Fig. 2). To evoke contraction in these fibers prolonged stimulation is necessary. That these contractions are not the result of incipient degeneration of the fiber is attested to by the fact that under the microscope these fibers appear completely undamaged and uniform and are capable of producing contractions for an indefinite length of time (2-3 hours) the same as fibers of the transitional and tetanic types. These muscle fibers evidently must belong to the tonic type.

The majority of fibers extracted from the white sole muscle, which is characterized by many authors as a fast dynamic muscle, are distinguished by fast high contractions not unlike the contractions of the

tetanic fibers of frog muscles. This type of contraction persists for all stimulation intensitites; with increasing stimulus strength, only the amplitude of contraction increases somewhat (Fig. 3). However, with very strong stimulation of these fibers there appears a small unstable plateau which evidently is a result of damage occurring within the fiber under the influence of an excessively strong stimulation. By the nature of their contraction these fibers evidently should be considered tetanic fibers. It should be mentioned that sometimes these fibers also occur in the heel muscle, especially in its deeper portions.

Therefore, during the course of our work we succeeded in extracting from skeletal muscles of the rat diverse muscle fibers, some contracting in a tetanic manner and others in a tonic manner. These facts enable us to adhere to the view that specialized muscle fibers are also present in mammals. In addition it appears that together with specialized tetanic and tonic fibers there are huge numbers in a transitional stage combining the ability to contract fast and tonically in varying degrees.

The distribution of muscle fibers according to their contraction characteristics evidently is represented by a normal curve, the extreme ends of which are occupied by specialized tetanic and tonic mechanisms; all the rest of the area is occupied by transitional mechanisms approaching in varying degrees either the tetanic or the tonic type.

SUMMARY

The problem of the presence in mammals of specialized muscular fibers adjusted to tonic or tetanic activity is debatable. Experiments were performed in order to investigate this question.

Single muscular fibers were isolated from the muscles of mammals. It was established that specialized muscular fibers, contracting tonically or tetanically are present in rat muscle. Fibers of the transitional type combining both functions were also found.

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MORPHOLOGY AND PATHOMORPHOLOGY

CERTAIN MORPHOLOGICAL ALTERATIONS IN ISOLATED PORTIONS OF THE INTESTINE AFTER THEIR DENERVATION

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When investigating the effects produced upon intestinal secretion by an interruption of the mesenteric nerves, one of the authors [2, 3] discovered that in dogs this "denervation" of portions of the intestine leads to pronounced alterations, both quantitatively in the volume of the secretion and in the qualitative composition of the enzymes present in the secretion.

In the present study our aim was to investigate the morphological alterations that take place in the mucosa and intramural nervous system of these portions and compare the results obtained with the known data on their secretory function.

The studies were conducted on dogs with two adjacent portions of the small intestine which had been isolated. One of the portions was operated following the method of Thiry, while the second was "denervated" by one of two methods: 1 to 5 months after having transplanted the portion under the abdominal skin of the animal, the nerve vascular bundle of this region was transected or else, while the isolated portion was being prepared, the visible mesenteric nerves were cut and the vessels were painted with a 5% solution of phenol. The intramural portion of the nervous system was maintained in these isolated bowel loops besides the fact that new nerves would grow into them so that the term, "denervated," really must be placed within the quotation marks as it is not true, strictly speaking.

Within 1-2 days following "denervation" by either method there took place a marked increase in the amount of the liquid volume of the secretion and also of the quantity of the solids in it so that "mucous clumps" were formed. However, the amount of enzymes produced per unit of time fell off markedly. This continued for 6-10 days. By the 12 to 20th day, the amount of the intestinal juice produced by the "denervated" portions fell to the level characteristic for Thiry fistulae. Then the secretion continued to diminish to levels 5-10 times lower and continued on this lower level all the time that these "denervated" transplanted subcutaneous intestinal segments continued to be observed. The segments which were allowed to stay within the abdominal cavity, after $2\frac{1}{2}$-3 months, would start to have an increase in the secretory levels although it never reached the levels characteristic for Thiry fistulae.

One to $1\frac{1}{2}$ years following the "denervation" 6 dogs were subjected to morphological studies. In 4 dogs biopsies were taken from the isolated portions, both Thiry and "denervated," while 2 dogs were sacrificed. Morphological studies were made on 4 dogs with isolated segments of the upper region of the small intestine while in 2 dogs the isolated segments were in the terminal portions of the ileum.

Snips from the isolated segments were placed in 12% solutions of neutral formalin. The mucous membrane stain was hematoxylin – eosin while the intramural nervous system was investigated by the use of the method of Bielschowsky-Gross.* Additionally, 3 dogs had two intestinal areas, one above and one below the operative site of the resection (as determined by the scar), subjected to microscopic studies.

EXPERIMENTAL RESULTS

Macroscopic investigation showed that the "denervated" intestinal segments, transplanted under the skin, atrophied markedly shrinking to half the original size. The diameter diminished in proportion. The "denervated" portions permitted to stay within the abdominal cavity macroscopically could not be distinguished from the usual Thiry fistula segments, the dimensions remaining unaltered.

Mucous membrane. Histological studies of the walls of the isolated segments operated by the Thiry method showed no pathological alterations. In these portions, as well as in the normally functioning intestine, the thickness of the mucosa, the frequency of the distribution of the villi and their dimensions were all the usual. The villous epithelium was high, prismatic; the cell nuclei were all on one level and the surface cupping was clearly outlined. The intestinal epithelium maintained the various stages of the secretory process [1]. The stroma of most of the villi was covered by an even layer of epithelium while on the sides of the villi the epithelium could be observed being gathered into folds or actually beginning to separate. The tops of some of the villi had some small tubular epithelial formations. In some areas between the villi much mucus, secreted by the goblet cells, could be observed gathered together. The mucosal epithelial layer itself was somewhat infiltrated by leucocytic elements. The intestinal crypts seemed to be built and distributed quite normally. The epithelium of the crypts evidenced mitotic figures (Fig. 1,a).

In the isolated portions, "denervated" by transections of the nervous twigs and allowed to remain within the abdomen, the mucous membrane looked hardly different from the mucous membrane lining the usual Thiry segments. Only in occasional places were there fewer villi and crypts. Within the epithelial layer there could be seen a greater number of goblet cells. The mucous membrane did not appear to exhibit other changes (Fig. 1,b).

In the isolated segments, "denervated" and transplanted subcutaneously, the epithelium evidenced severe atrophy. It was much thinner than the segments isolated by the Thiry method and permitted to remain within the abdomen. The shape of the villi altered markedly, the form becoming irregular and the number much less. The intestinal crypts became less frequent and more shallow at the same time. There was an increase in the quantity of interstitial connective tissue within the epithelial layer itself. The surface of the mucous membrane of the isolated segments of the lower portions of the small intestine became covered with cubical epithelium. In some areas of the epithelium the covering became uneven. In sections taken from the upper regions of the small intestine the epithelium, while remaining comparatively high, developed unequal cells of varying heights having uneven apices, the surface indentation being sometimes absent and the cell edges losing their sharp borders. There was no evidence of epithelial accumulation on the surfaces of the villi and only occasional villi had a little exfoliation of the epithelium (Fig. 1, c). The smooth muscle fibers within the epithelial layer and within the external muscular layer of the "denervated" sections which had been transplanted subcutaneously frequently displayed wrinkled, pycnotic nuclei.

Nerve plexi. In the isolated Thiry segments the intramural plexi, both the Meissner and the Auerbach, are difficult to distinguish from the intramural ganglia to be observed in the wall of a normally functioning intestine. Within the segments both the plexi have a larger number of ganglia having a variable number of nerve cells but their bodies appeared to have normal contours and the usual variation in the degree of impregnation from light to much darker. The nuclei were rounded, homogeneous, having nucleoli and the submucous plexi being darker than the cell bodies. The nerve cells within the ganglia were of various dimensions and varying stages of differentiation. The glial content of the nerve ganglia did not differ from the usual (Fig. 2,a).

Within the "denervated" intestinal segments left within the abdominal cavity, the intramural nerve system showed a few changes when compared with the nervous system observed within the isolated Thiry segments. Along with a large number of ganglia having a normal structure, there were encountered neurons manifesting a marked nuclear ectopia. Many nuclei and also the protoplasm had clumps of dark material heavily impregnated with silver as well as vacuoles. Within the Auerbach plexi there could be seen dying neurons with pycnotic nuclei and a vacuolated protoplasm. The nerve fibers seen in the plexi of segments, both "denervated" and

*Our microscopic studies were aided by consultations of E. A. Rudik and S. I. Matveeva to whom we extend our gratitude.

left within the abdominal cavity as well as within the plexi of the Thiry preparations, could not be really differentiated from those seen in the walls of normally functioning intestine (Fig. 2,b).

Especially marked changes were to be observed within the intramural nervous system of the "denervated" segments which had been transplanted subcutaneously. Practically all the ganglia of the Auerbach plexus and the majority of those in the Meissner plexus had, along with the normal neurons, nerve cells showing degeneration and destruction. In the Meissner plexus of these segments the nerve cells were smaller than in the usually innervated intestinal portions, the neuronal nuclei were rounded, intensively impregnated or having angular shapes. Nucleoli could not be seen in all the nuclei. Around such nuclei could be seen cell debris of uncertain forms. In the Auerbach plexus there could be seen frequently neurons with nuclei lying at the cell periphery, the center and edges being taken up by large vacuoles. Cell shadows without nuclei could also be seen. Many cells were pycnotic and inordinately impregnated with silver. At times, remnants of such cells were encountered. In occasional ganglia of both plexi but especially Meissner, there would be seen just 3-5 sharply altered nerve cells or only satellite material (Fig. 2, c). Thus, when the physiological and morphological alterations in the isolated intestinal segments, whether "denervated" by various methods or left innervated, are compared, there is evident complete correlation between the morphological changes and the functional capabilities of these segments.

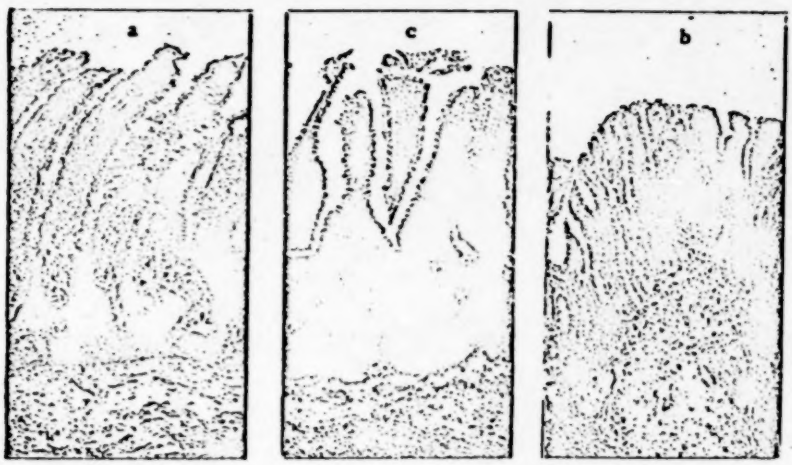

Fig. 1. Mucous membrane of isolated intestinal segments. Stained with hematoxylin-eosin. 60 fold magnification.
a) Isolated segment of an upper portion of the small intestine operated by the Thiry method, the nervous system remaining intact; b) same but "denervated" and allowed to remain within the abdominal cavity; c) isolated section of the terminal ileum, "denervated" and transplanted subcutaneously.

The morphology of the segments prepared by the Thiry method revealed no significant deviations from the structures seen in the normally functioning intestine of healthy animals. The secretory capacities of these segments were observed for $3\frac{1}{2}$-4 years and they remained well-expressed and constant. The "denervated" segments, after the sectioning of the mesenteric nerves, displayed a marked secretory disturbance. At first, "denervation" by both methods gave similar results. These, apparently, are the consequence of the removal of the trophic controls which had been arriving from the central nervous system. Following 1-2 months, the secretory capabilities within the segments tended somewhat to revert to normal but full restoration was observed only within the segments that had been permitted to remain within the abdominal cavity.

There are grounds for believing that new nerve fibers grow into the "denervated" intestinal segments, this leading to partial restoration of the trophic influences of the central nervous system. This restoration of

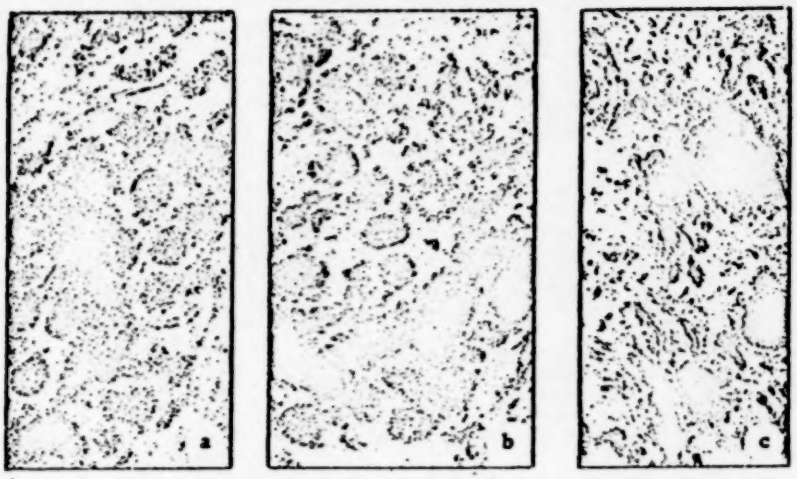

Fig. 2. Nerve ganglia of the submucous plexi within the isolated bowel segments.
Stained according to Bielschowsky-Gross. Magnification 550 x.
a) Isolated Thiry segment; b) "denervated" segment left within the abdominal cavity; c) "denervated" intestinal segment transplanted subcutaneously.

connections with the central nervous system is suggested by the fact that examination of the mesentery distal to the site of sectioning of the nerves reveals nerve fibers of normal structure and, apparently, quite functional. The extent of the return of the secretory function and the severity of the degenerative processes observed within the "denervated" intestinal segments appears to depend primarily on the character of the newly growing nerve fibers.

Into the intestinal segments transplanted to the subcutaneous region, the nerve fibers that grew in came from subcutaneous nerves which usually have no digestive associations. It is these segments that showed the heaviest morphological evidence of atrophy. Their capacity to secrete enzymes remained severely depressed.

Into the "denervated" segments which had been permitted to stay within the abdominal cavity there occurred an ingrowth of nerve fibers from nerve trunks innervating the digestive organs. These segments showed a much lesser degree of morphological evidence pointing to atrophy. Their secretory function tended to revert almost to normal, being much greater than in the transplanted segments even though less than what was seen in the segments prepared by the Thiry method only.

Our data all point to the powerful trophic effect exerted upon the intestine by the central nervous system by way of the mesenteric nerves. It would appear that the central nervous system regains control through the medium of the ingrowing nerve fibers and that its trophic influences are much more effectively displayed by way of nerve fibers normally innervating the contents of the abdominal cavity than by way of nerve fibers coming from the subcutaneous nerve net.

SUMMARY

Isolated intestinal segments were prepared in dogs. One segment was made by the usual Thiry method. The other was additionally "denervated" by one of two methods and then, either left within the abdominal cavity, or else transplanted subcutaneously.

The morphological and physiological alterations produced were observed over a period of several years.

The central nervous system exerts a powerful trophic effect upon the intestine. The "denervated"

segments are restored to central nervous system control by ingrowth of new nerve fibers. In the segment that had been left within the abdomen, these fibers come from nerves which normally innervate the digestive organs. The transplanted segment receives nerve fibers from the subcutaneous net. It is an experimental fact that the intra-abdominal segments tend to return to normal to a much greater extent than do the segments transplanted under the skin.

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CEREBELLAR ARCHITECTONICS OF THE BLOOD VESSELS FOLLOWING EXPERIMENTAL LIGATION OF THE COMMON CAROTID ARTERIES IN RABBITS

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The morphological alterations of the arterial stream which occur in various divisions of the brain following the exclusion of individual main cervico-cerebral vessels have not so far been studied very thoroughly, although there exist a number of studies in this field [1, 2, 3, 4, 6].

The present investigation had as its goal the study of the changes occurring in the cerebral arteries of the rabbit after a simultaneous bilateral ligation of the carotid arteries.

EXPERIMENTAL METHODS

Our studies were conducted upon 14 adult rabbits of both sexes. All the animals were operated under ether narcosis. Both the common carotid arteries were ligated. Externally, during the postoperative period the animals could not be distinguished from unoperated rabbits as no functional disturbances of any kind could be observed; they were all sacrificed from 7 to 265 days following the ligations. The arterial system of the head and neck of each animal was injected with a contrast medium (3 parts of lead white plus 1 part of carbon black stirred into turpentine to a creamy consistency), the thoracic aorta being the portal of entry, after which the brain was removed from the skull case and hardened first in 5% and then in 10% formalin solution. The hardened preparations were first examined for their external vessels with the aid of a binocular lens and then histological sections 200, 300 and 400 μ thick were made in the frontal, sagittal and horizontal planes so that the intraorgan arteries could be studied. The diameters of the principal arteries were determined with the aid of a micrometer objective and ocular. For a control comparison we studied the corresponding vessels of the region being examined utilizing the same technique in 10 adult rabbits.

EXPERIMENTAL RESULTS

Our observations indicate that simultaneous ligation of both common carotid arteries leads to a considerable reconstruction of the arteries to be found at the base of the brain. This last, in particular, expresses itself by an alteration of the angle at which the basilar artery terminates by dividing into the 2 caudal or posterior cerebral arteries, the normal acute angulation becoming blunted so that, as early as the 14th postoperative day, this obtuseness is evident while by the 128th day the angle reaches 180°. Simultaneously with this alteration the diameter of the indicated arteries increases; thus, the diameter of the basilar artery by the 30th postoperative day reaches 1.3 mm while by the 220th day it becomes 2 mm, i.e., twice the normal dimension.

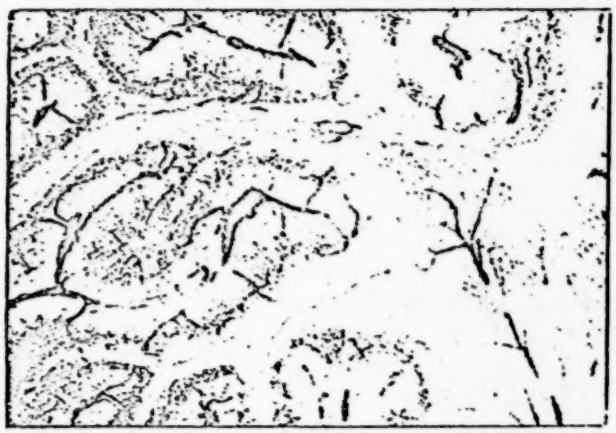
Along with the alterations of the individual components of the circulus arteriosus (Circle of Willys), analogous manifestations govern the compensatory reconstruction of the arterial bed of the cerebellum. Thus the nasal or superior cerebellar arteries which branch from the posterior cerebral arteries present the appearance of being direct continuations of the latter. This occurs because the angle of emergence of the superior cerebellar arteries is changed from the normal acute to a blunt angle; simultaneously, these arteries increase in diameter by 0.2-0.3 mm. The formation of tortuous collaterals upon the cerebellum must be considered as

rather rare. It was noted that the caliber of the branches leaving the superior cerebellar arteries to supply the midbrain also increased.

The caudal or inferior posterior cerebellar arteries increase their diameter in the initial period of compensatory widening of the vessel bed and maintain this later by exceeding the normal diameter of 0.2-0.3 mm by 0.1-0.15 mm. It should be noted that the cerebellum of rabbits is supplied only by two pairs of cerebellar arteries. The third pair, the inferior posterior cerebellar arteries, which is present in man, dog, cat and others is absent in the rabbit according to the data of A. Tschernyscheff and J. Grigorowsky [5] as well as our own observations.

The arterial net of previously existing cerebellar anastomoses seen in preparations made in animals sacrificed shortly after the operation is distinguished by considerable density; at later periods this net is less dense but the anastomoses of the individual collaterals are much more distinct.

The intracerebellar arteries react to the ligation of the carotid arteries in the same manner as do the external. The diameter of the sulcal arteries by the twenty-first lay postoperative day reaches 100 μ as against a normal of 60-70 μ. The long and short cortical branches arising from the sulcal arteries widen their diameter by 10-20 μ, the normal diameter within the short branches fluctuating between 25-30 μ, while within the long, the fluctuation is 35-45 μ. The increase in the diameters is to be seen mostly in those branches which go off at an acute angle.



Intracerebellar arteries on the 220th postoperative day following the exclusion of both common carotid arteries. Magnification 15x.

Finally, it should be noted the increase in the diameter of the collaterals and the greater density of the arterial net within the cortex of the cerebellum leads to the erasure of the usually well defined boundaries between the vessels of the individual layers. This is seen first in the vessels of the cerebellar cortical ganglionic layer. Within this layer normally the short cortical branches divide arc-wise forming a system of arterial anastomoses shaped like arcades. Under the conditions of the experiment the individual short cortical branches penetrate more deeply into the granular layer forming irregular branchings as a result of which the arcade-like system of anastomoses no longer exists. Similar findings were noted by N. A. Dzhavakhisvili-Komakhidze [1] who studied the vessels within the cruciate sulcus in the dog and by V. P. Kurkovskii [3] who investigated the cerebellar cortex of the white rat, this latter author taking the position that this is the cause of the special sensitivity displayed by the ganglionic layer of the cerebellar cortex to acute anoxemia.

Thus it can be seen that bilateral ligation of the common carotid arteries produces the development of collateral circulation in the cervical region and produces also considerable alterations in the angioarchitectonics of the brain as a whole and the cerebellum in particular.

SUMMARY

In order to study the architectonics of the cerebellum in conditions of collateral circulation, simultaneous bilateral ligation of the common carotid arteries was carried out in 14 rabbits. No functional disturbances were noted in animals during the postoperative period. External arteries were studied on injected preparations of the cerebellum, while the internal arteries were examined on the total histological sections. When the bloodflow is disturbed in these vessels, there is a morphological change in the arteries of the brain and cerebellum. With time these morphological changes develop further. The angles of division, origin and fusion of various blood vessels is changed, their diameter increases, and their course becomes tortuous. The same changes take place in the internal cerebellar arteries i.e., sulcal arteries and short and long cortical branches which originate from them. There is modification in the vascular structure of the separate layers in the cerebellar cortex, as a result of which the borders between them become indistinct.

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METHODS

ATTACHMENT TO ELECTROCARDIOGRAPH ALLOWING SIMULTANEOUS RECORDING OF E. C. G. AND RESPIRATORY MOVEMENTS•

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Given the appropriate apparatus, simultaneous recording of the electrocardiogram and respiration presents no serious difficulty. For this, however, either a string or a cathode ray oscillograph is necessary, and then both electrocardiogram and pneumogram can be recorded one above the other by various methods which have been described by Kh. B. Gurevich [1], D. N. Menitskii [2], L. I. Shvang and A. D. Fedorov [3] and others.

As far as we know, no device for simultaneous registration of both curves on the widely used EKP-4M electrocardiograph has been described.

Together with F. T. Shevchenko of the radio section of the Tuberculosis Institute we have developed a simple addition to the electrocardiograph which allows the respiration curve to be recorded together with the electrocardiogram on the same strip of paper. The device which is shown in Fig. 1 consists of three parts: 1) a Marey's capsule fitted with a taut fine rubber membrane and bolted to a stand which is integral to the case of the electrocardiograph; 2) a lever bearing a mirror so arranged that the axis of the lever is free to rotate between the pointed ends of two bolts; 3) the illumination system.

A short length of rubber tubing is joined to the tube leading from the capsule and is led out from the apparatus through a hole drilled in the front folding side of the electrocardiograph. This tube is connected via a three-way tap to the respiration pressure transmitter. The latter consists of a 20 cm length of corrugated rubber tubing 3.5 cm in diameter.

The whole air system may be either completely enclosed, or it may be connected to the atmosphere by a small glass tap. The latter must be open when the pressure transmitter is placed in position.

A light metal disk 5-6 mm in diameter is fixed to the membrane of the capsule and is joined to a length of fine wire. The latter connects the membrane with the arm of lever "b". A mirror "c" in a metal mount is fixed to the other arm of the lever. Close up against the mirror there is the lamp housing "d" in which is drilled a 0.1 mm diameter hole through which passes a narrow beam of light from the lamp, mounted in a focussing tube (the tube support can be seen in Fig. 2).

The lamp and mirror are disposed so that the reflected bundle of rays passes through a lens (Fig. 1, f) onto the light-sensitive strip. The spot focussed by the lens falls on a line dividing the upper and middle thirds of the strip, so that when the light spot from the galvanometer has been slightly decentered, and when tension is applied to the strip, the pneumogram and electrocardiogram are recorded one below the other.

The position of the light spot can be controlled together with the galvanometer spot, since part of the beam reflected from the inclined mirror (Fig. 1,c) of the electrocardiograph falls on the inspection window (Fig. 1, e).

• Read on the June 20, 1957 at a Joint meeting of the staff of the laboratory of respiration and circulation of the Physiological Institute of the AN Ukrainian SSSR and the pathophysiological Laboratory of the Ukrainian Tuberculosis Institute.

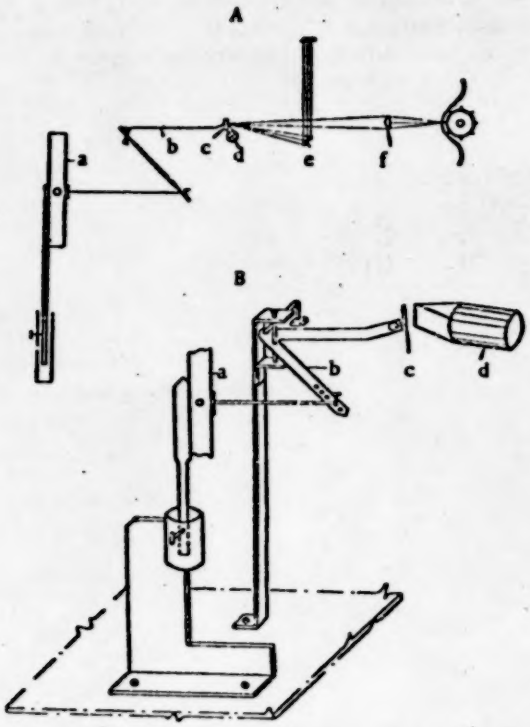

Fig. 1. (A) Diagrammatic (B) perspective drawings of the attachment of the EKP-1M electrocardiograph. Description in text.

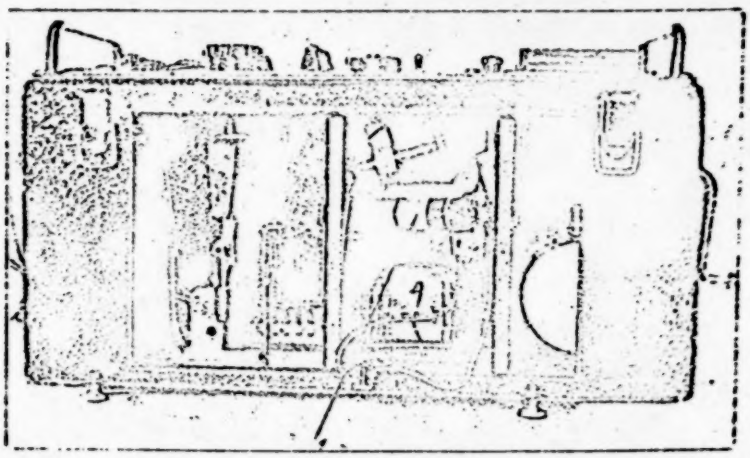

Fig. 2. General view of the electrocardiograph with attachment.

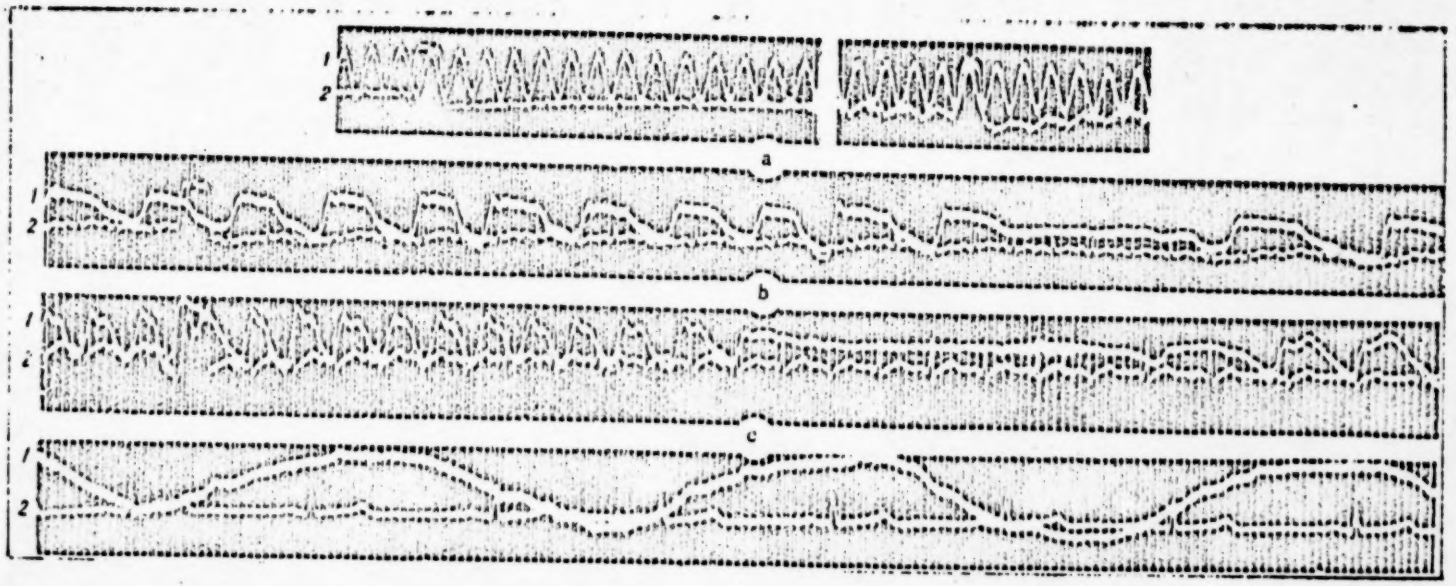

Fig. 3. Examples of simultaneous recordings of pneumogram and electrocardiogram.
a) Pneumogram and electrocardiogram of a rabbit (leads I and II); b and c) the effect of intravenous injection of cititone (0,5 ml diluted 1 : 5) on the respiration and heart rate of rabbits (thoracic ECG lead); millivolt mark – time of injecting solution into peripheral ear vein; d) electrocardiogram (ECG lead II) and human pneumogram; 1) pneumogram (movement of curve downwards represents inspiration); 2) electrocardiogram.

Vacant space in the electrocardiograph is used for mounting the parts of the apparatus just described. The capsule and the lever bearing the mirror are placed in the galvanometer section; the illumination system is placed by that belonging to the E. C. G. (Fig. 2). The lamp for recording the pneumogram is fed from the E. C. G. batteries. Provision is made for switching the lamp on or off. The switch is brought to the outside and fixed on the front wall.

The pneumogram is recorded as follows. After the pressure transmitter has been attached to the chest or abdominal wall of the animal, the tap connecting the system with the atmosphere is closed. After this, pressure changes caused by respiratory movements are transmitted to the rubber membrane of the capsule, and from there through the lever to the mirror. When the light is switched on, oscillations of the mirror cause a corresponding displacement of the light spot on the recording strip. The amplitude of the mirror oscillations, and consequently the amplitude of the film trace of the respiratory movements can be controlled by choosing an appropriate thickness of the capsule membrane, and by placing the screw (Fig. 1, B) in the appropriate hole in the arm of the lever "b". Also it must be noted that various pneumatic pressure transmitters can be used depending on the particular requirements.

To record changes in response to stimuli which may cause considerable changes in the amplitude of the respiratory movements, an additional air-filled reservoir communicating with the transmitter and capsule should be fitted. The connection between these vessels allows control over the effective volume of air.

Figure 3 shows examples of simultaneous recordings of pneumogram and electrocardiogram.

If the speed of the recording strip is made sufficiently high, the 0.05 second interval marker in the apparatus allows accurate measurements of respiration frequency as well as the duration of the separate phases to be made even when the respiration rate is several hundred per minute (Fig. 3,a).

The millivolt knob on the electrocardiogram may be used to indicate the time at which the stimulus is applied so that changes in heart rate and respiration in response to various agents can be investigated. Curves b and c show the electrocardiogram and pneumogram before and after injecting 1 : 5 cititone* into the veins of the ear. The greatest change occurs in the respiration, and after a few seconds an apnea of varied duration takes place. Changes in heart rate are less marked, though the curves show a slowed rhythm.

We have used this device to investigate circulation time in rabbits (by measuring the time of onset of respiratory changes following injections of cititone).

The pneumogram and electrocardiogram can be recorded together not only in animal experiments, but in investigations of human subjects (Fig. 3,d).

Simultaneous recording of the two curves may find application in clinical practice in examining patients with cardiovascular and respiratory disturbances. Recording the respiration either by itself or together with the electrocardiogram extends the scope of the diagnosis, while the method is simple and demands no more time than does the normal electrocardiograph.

There is also the possibility that this method may be used to investigate other phenomena where the pneumatic recording technique is applicable.

SUMMARY

A simple device attached to the electrocardiograph EKP-4M which allows registration of the curve of respiratory movements simultaneously with electrocardiography is described.

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*As in original.

COMPLEMENT FIXATION TO A 50% HEMOLYSIS END POINT WITH PROTEIN ANTIGEN ADSORBED ON PAPER, AND ITS ACTION ON ANTI-CANCER SERA

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The quantitative complement fixation test (CFT) to 50% hemolysis end point for soluble antigens was developed in Heidelberger's laboratory [5], and later modified by Konikov [3]. We first used this method in our laboratory in 1955 to determine the serological activity of azoantigens and native proteins firmly fixed on chromatography paper [1, 2].

In the present paper we described the quantitative CFT in terms of the 50% hemolytic unit, using "paper" antigens, as suggested by V. S. Gostev who directed the investigation.

EXPERIMENTAL METHODS

Use of the 50% unit in CFT demands a strict control over all reagents and experimental conditions. In the antigen, the protein nitrogen is found by Conway's method, and the erythrocyte weight for the hemolysis is standardized with a photoelectric colorimeter. The complement is first titrated, and enough is then taken so that an excess remains both in the control and in the experimental tests.

In each case the excess complement is back titrated. From the difference, the number of combined complement units is found. A complement unit is taken as the amount of complement (in 0.5 ml) which will cause 50% hemolysis in 30 minutes at 37°C of 0.5 ml of the standard suspension of erythrocytes.

The reaction consists of two stages. Stage I, which consists of the actual combination of the complement with the specific antigen-antibody complex, takes place in the cold. A definite weight (15-20 mg) of antigen adsorbed on paper is placed in 0.3 ml of physiological saline, and 0.2 ml of antiserum diluted 1 : 10, and 0.1 ml of complement (either undiluted or diluted 1 : 2, according to its strength), are added. The total volume of the mixture in Stage I is 0.6 ml. Control of the antigen, antiserum and complement is essential.

Stage II consists of the back titration of the uncombined complement. On the following day a definite volume (usually from 0.01 to 0.1 ml) is taken from each experimental and control tube, it is made up to 0.5 ml with physiological saline, and an equal volume (0.5 ml) of a suspension of red cells is added. The mixtures are then placed for 30 minutes in an incubator at 37°C. The tubes are then cooled, centrifuged, and the coefficient of extinction of the supernatant fluid is determined using a photoelectric colorimeter. The percentage hemolysis is read from a graph of percentage hemolysis against the coefficient of extinction. In order to find the number of units of free complement in the sample, an amount of the mixture must be taken which results in a hemolysis of between 20 and 80% of the cells.

The amount of complement giving 50% hemolysis cannot usually be determined directly. It can, however, be calculated from Krogh's formula from the amount of complement causing a known amount of hemolysis:

$$\log x_2 = \log x_1 - n\log \frac{y}{100-y},$$

where x_2 is the amount of complement required to give 50% hemolysis; x_1 is the amount of complement taken in the experiment; n is the coefficient of extinction, which for the particular experimental conditions was equal to 0.2; and y is the percentage of hemolysis observed.

The number of units of complement in the sample are found from the 50% end point titration value. The amount of complement fixed is found by subtracting the number of units of free complement remaining in the mixture from the number in the control tube. The anticomplementary action of the antigen or antiserum may cause the number of free units of complement in the control tube to be less than the number used in the experimental tube. The calculation is based on the control tube showing the strongest anticomplementary effect. In cases where the anticomplementary effect of antigen or antiserum is very high it is best to increase the amount of complement somewhat, though excessively large amounts may reduce the accuracy of the test.

We used the method described above to test the serologic activity of several groups of "paper" antigens.

Preparation of "paper" azoantigen. Tissue extracts of a human stomach cancer and a normal stomach and spleen were made by triturating with ten times the volume of physiological saline, centrifuging, and then adding the azo dye diazodianizide.

The resulting azoprotein was adsorbed on strips of chromatographic paper, dried at room temperature, washed in water for 5-6 hours, and again dried at room temperature. The incorporation of diazodianizide into the protein molecule causes it to be fixed firmly to the paper.

Preparation of "native" antigen adsorbed onto paper. Strips of chromatography paper are immersed for 10-15 minutes in the saline extracts of human tissue. The paper impregnated with protein is dried in air at room temperature, then washed in tap water for 3-4 hours and again dried at room temperature.

The second method of preparing the paper and antigen is simpler and causes less denaturing of the protein.

The antisera used in the above experiments were obtained by immunizing rabbits by injecting macerated human stomach cancer intraperitoneally.

EXPERIMENTAL RESULTS

Table 1 shows a typical example, which is one of the 40 experiments using paper azoantigen and the 50% end point.

TABLE 1

"Paper" azoantigen	Control antigen	Rabbit serum No. 1831		Normal rabbit serum		Control	
		Units of complement					
		free	combined	free	combined		
Human stomach tissue	30	5,5	24,5	30	—	Diazodianizide treated paper	30
Human splenic tissue	30	6	24	30	—		
Human serum globulin	30	—	30	30	—	Pure chromatography paper	30
						Rabbit serum No. 1831	30
						Normal rabbit serum	30
						Complement	30

Note: Serum No. 1831 is antihuman gastric carcinoma. The figures indicate the number of complement units.

These results show the marked serological activity of the "paper" azoantigens of tissues from the human stomach and spleen and the serum globulin; these combine specifically with a considerable amount of complement in the presence of the corresponding antiserum. In this particular case, anticarcinoma serum was used. The specific combination of the complement with normal tissue proteins in the presence of anticarcinoma antiserum is due to a common antigen present in normal and cancerous human subjects.

Many experiments were also carried out to determine the serological activity of "native" "paper" antigens.

The results of one of these experiments are shown in Table 2.

TABLE 2

Antiserum		"Paper" tissue antigen					
		of gastric carcinoma		of normal stomach		of spleen	
		units of complement					
		free	combined	free	combined	free	combined
Serum No. 3804		4.1	28.9	4.1	28.9	4.1	28.9
" No. 2124		3.8	29.2	4.1	28.9	3.8	29.2
" not containing Antibody		33	—	33	—	33	—
Control	Antigen	33	—	33	—	33	—
	Serum No. 3804	33	—	—	—	—	—
	Serum No. 2124	33	—	—	—	—	—
	Serum of normal rabbit	33	—	—	—	—	—
	Complement	33	—	—	—	—	—

Note: Sera Nos. 3804 and 2124 are rabbit antihuman gastric carcinoma sera. The figures indicate the number of complement units.

It can be seen from Table 2 that the "native" antigens, fixed by drying on chromatography paper, show a high activity in their action on polyvalent antihuman gastric carcinoma antiserum.

Comparative experiments showed that "paper" azoantigens are not superior to "native" "paper" antigens. On the contrary, "native" "paper" antigens are not only highly active serologically but they are less easily denatured, and this enables them to be preserved unchanged.

The antigens adsorbed onto paper preserve their power to react with serum for several months.

Our results show that complement fixation tests to 50% end point can be made on antigens adsorbed on paper. Fixation of complement to 50% end point with the paper antigen not only gives an accurate quantitative result, but has the further important advantage that the antigen adsorbed on paper may be readily removed from the mixture and replaced by a different antigen of known immunological specificity as required by the particular investigation.

V. S. Gostev suggested that we should use this method to attempt a specific extraction of antibodies so as to obtain antisera with a narrow range of specificity.

We give here the preliminary results which we have obtained. For this extraction we took immune sera obtained from rabbits by immunizing with ground up human gastric carcinoma given intraperitoneally. "Native" "paper" antigens from normal and cancerous human stomachs were used as antigens.

TABLE 3

Extraction of Antihuman Gastric Carcinoma Sera by Normal Stomach Protein Adsorbed on Paper (Experiment No. 12 of 2/4/1957. Total number of experiments equals 18)

Stage I of the reaction in the cold		Sample	1	2	3	4	5	6	7	8	9	10	11
	1st day	Antiserum in ml	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
		Physiological saline in ml	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0 3
			—	—	20 mg Ns 1 day	20 mg Ns 2 days	20 mg Ns 1 day	20 mg Ns ½ day, 20 mg Ns ½ day	—	20 mg Ns 1 day	20 mg Ns 2 days	20 mg Ns 1 day	20 mg Ns ½ day 20 mg Ns ½ day
	2nd day	'Paper' antigens	—	—	—	—	20 mg Ns 1 day	20 mg Ns 1 day	—	—	—	20 mg Ns ½ day, 20 mg Ns ½ day	20 mg Ns ½ day 20 mg Ns ½ day
	3rd day		—	20 mg Ns	20 mg Ns	20 mg Ns	20 mg Ns	20 mg Ns	20 mg Canc s	20 mg Canc s	20 mg Canc s	20 mg Canc s	20 mg Canc s
		Complement in ml	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1

Stage II	4th day	Titration of uncombined complement																					
		1st-sample		2nd-sample		3rd-sample		4th-sample		5th-sample		6th-sample		7th-sample		8th-sample		9th-sample		10th-sample		11th-sample	
Units of complement		free	combined	free	combined	free	combined	free	combined	free	combined	free	combined	free	combined	free	combined	free	combined	free	combined	free	combined
Serum No. 2124		51	—	—	51	5.3	45.7	6.4	44.6	51	—	51	—	5.1	45.9	5.5	45.5	5 3	45.7	6	45	7.3	43 7
Serum No. 3017		51	—	5.5	45 5	5.8	45.2	6.6	44.4	51	—	51	—	5.3	45.7	6	45	6	45	6.2	44.8	6.6	44.4
Serum of normal rabbit		51	—	51	—	51	—	51	—	51	—	51	—	51	—	51	—	51	—	51	—	51	—
CSe No. 2124		51	—	CSE of normal rabbit				51	—	Paper alone + serum No. 3017				51	—	Control complement		51	—	—	—	—	—
CSE No. 3017		51	—	Paper alone + serum No. 2124				51	—	Paper alone + serum of normal rabbit				51	—	—	—	—	—	—	—	—	—

Note. Ns — normal stomach; Canc s. — cancer of stomach; CSe — control serum. Numbers in Stage II of reaction indicate number of units of complement.

Extraction of the anticancer serum was carried out in the quantitative CFT as follows.

Eleven samples were taken. To each was added 0.2 ml of antiserum diluted 1:10 and 0.3 ml of physiological saline (Table 3).

Stage I of the reaction lasted up to 3 days: the normal stomach "paper" antigens were replaced, as shown, in samples 1 to 11 for the first 2 days, in order to extract the antibodies to normal stomach antigens. On the third day, in samples 1 to 6, the "paper" antigen of normal stomach was replaced by fresh normal stomach antigen (control to show complete extraction), and in samples 7-11 the normal stomach antigen was replaced by gastric carcinoma antigen, as a control on the specificity of the extraction. No further antigen was added to sample 1, and the second and seventh samples served as controls over the immunological activity of the "paper" antigens. 0.1 ml of whole complement was added together with the last portion of antigen. Next day, after titrating the complement, the uncombined complement was calculated by the method described above.

It can be seen from the results given in Table 3 that complete removal of the antibodies to normal stomach tissue antigens occurs as a result of leaving the mixture in contact with these antigens for two days. In this case, 20 mg of antigen is placed in the test tube on the first day, and is then replaced by more of the same antigen. Sera which have been exhausted in this way do not react with normal gastric tissue antigens (tests 5 and 6), while with gastric carcinoma antigen (tests 10 and 11) they combine with a considerable number of complement units, which demonstrate clearly the specific nature of the antibody depletion.

These results show that protein antigens adsorbed on paper have a specific effect in depleting antisera, and that the effect can be measured quantitatively by CFT.

Thus, the 50% end point quantitative CFT demonstrates a high serological activity in protein antigens fixed on paper either as azocompounds or by simple drying at room temperature. The method allows various antigens to be used one after the other in the course of a single experiment. This permits proteins adsorbed onto paper and the quantitative CFT to be used to determine the optimal conditions for obtaining antisera with a narrow range of specificity.

SUMMARY

Protein antigens fixed on paper in the form of azocompounds or by simple drying at room temperature have a high serological activity and specifically fix the complement in the presence of a corresponding antiserum. This was established by the method of quantitative reaction of complement fixation (50% titer). Complement fixation test (50% titer) for "paper" antigens gives an opportunity of using different antigens in the same test and replacing them during the experiment. This permits the use of antigens adsorbed on paper and the quantitative complement fixation test for investigation of the optimal conditions of obtaining the specific antisera.

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* In Russian.

** Original Russian pagination. See C. B. Translation.

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CONTENTS

PHYSIOLOGY

	Page	Russ. page
Analysis of Cutaneous Reception of Pressure in Man Using the Method of Conditioned Vascular Reflexes. Communication IV. The Effect of Various Pharmacologic Substances on the Character of Conditioned Vascular Reactions Developed to Barostimulation of the Skin. R. A. Fel'berbaum	263	3
Participation of the Nervous System in the Action of Secretin. O. G. Onisko	268	7
Comparative Data on Changes in Higher Nervous Activity in Dogs with False and True Pregnancy. V. Ia. Katinas	272	11
Subordinating Effect of the Diencephalon on Accommodation of the Sciatic Nerve. V. S. Khadzhikov	276	15
The Mechanism of Insulin Action. Communication V. Effect of Insulin on Blood Glucose Content in Dogs During Electrosleep. S. V. Zakharov and Iu. S. Tatarinov	281	19
The Role of Hyaluronidase in the Process of Urinary Excretion. L. N. Ivanova	285	22
The Role of Baroreceptive Systems in the Nasal Cavity of Birds in Regulation of Respiration. Communication II. A. B. Vishnepolskii	290	27 ✓
Redistribution of the Blood in the Blood Vessels of Lower Monkeys. M. I. Kuksova	295	31
The Effect of Vitamin C on the Absorption of Glycine and Chlorides by the Intestine. I. Ia. Nekachalova	299	35
Tonic Reflexes in Man. Communication II. Effect of Unstable Position of the Body on the A. A. Ukhtomskii Tonic Reflex. A. S. Dmitriev	303	38

PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

Serum Aldolase Content of the Blood of Dogs with Experimental (Myocardial) Infarction. E. I. Chazov and M. M. Savina	305	41
Role of Interoceptors in the Regulation of Oxygen Saturation of Arterial Blood. Communication III. Effects of Denervation of the Carotid Sinus Region on Oxygen Saturation of Arterial Blood and on Pulmonary Ventilation. N. N. Beller	309	45 ✓
Effect on Renal Function of Hemisection of the Spinal Cord of Dogs. Communication II. Effects on Renal Function of Repeated Hemisection of the Spinal Cord in the Cervical Region. V. F. Askerov	313	48
Absorption of Radioactive Iron, and Its Assimilation by Erythrocytes in Anemia Caused by Partial Denervation of the Stomach. K. S. Zamychkina and R. A. Durinian	317	51

	Page	Russ. page
Effect on Capillary Permeability of Single and Repeated Overheating of an Organism. F. F. Sultanov	322	56
Effect of Some Bacterial Intoxications on the Cholinesterase Activity of Rats' Brain. E. I. Pavlov and V. S. Zueva	326	60
BIOCHEMISTRY AND BIOPHYSICS.		
The Mechanism of "Paradoxical" Increase of Blood Diaminoxidase (Histaminase) Activity Under the Influence of Dimedrol and Thiamine. L. P. Kluga	330	64
Dependence of the Phagocytic Reaction of Leukocytes on their Carbohydrate Metabolism. O. S. Sherstneva	333	67
PHARMACOLOGY		
The Antishock Effect of Aminazine and Mepazine. L. N. Sinitsyn	336	70
The Competitive Character of the Action of Ganglion Blocking Agents – Derivatives of Quaternary Ammonium Compounds. M. L. Tarakhovskii	339	72
MICROBIOLOGY AND IMMUNITY		
A Study of the Bacteriostatic and Chemotherapeutic Activity of the New Drug Cycloserine. M. A. Breger, B. Ia. Stukalova and V. S. Mitrofanov	343	77
The Detection of Tissue Antigens. Report No. III. The Comparative Sensitivity of the Anaphylaxis Reaction and Serological Investigation in the Detection of Small Doses of Antigen. V. I. Ioffe, S. A. Anatolii and L. P. Kopytovskaia	347	80
The Excretion of Dysentery Antigens by the Kidneys in Dogs with Unchanged and Changed Immunological Reactivity. A. A. Pol'ner	352	85
The Incubation Period in Passive Immunity to Tetanus Toxin. G. N. Kryzhanovskii	357	91
ONCOLOGY		
The Effect of Extracts of Hippophae rhamnoides Upon the Development of Tumor Transplants in Animals. E. Ch. Pukhalskaia	363	97
EXPERIMENTAL BIOLOGY		
The Presence and Distribution of Alkaline Phosphatase in the Organ of Corti in Animals in a State of Relative Rest and During Auditory Stimulation. Ia. A. Vinnikov and L. K. Titova	367	101
The Problem of Specialized Muscle Fibers in Mammals. G. N. Chetverikov	373	107
MORPHOLOGY AND PATHOMORPHOLOGY		
Certain Morphological Alterations in Isolated Portions of the Intestine after Their Denervation. N. A. Nilova and L. S. Fomina	376	110
Cerebellar Architectonics of the Blood Vessels Following Experimental Ligation of the Common Carotid Arteries in Rabbits. V. V. Stenyk	381	114

	Page	Russ. page
METHODS		
Attachment to Electrocardiograph Allowing Simultaneous Recording of E. C. G. and Respiratory Movements. L. P. Cherkasskii	384	118
Complement Fixation to a 50% Hemolysis End Point with Protein Antigen Adsorbed on Paper, and Its Action on Anti-Cancer Sera. N. A. Shagunova	388	122

ABBREVIATIONS MOST FREQUENTLY ENCOUNTERED IN RUSSIAN BIO-SCIENCES LITERATURE

Abbreviation (Transliterated)	Significance
AMN SSSR	Academy of Medical Sciences, USSR
AN SSSR	Academy of Sciences, USSR
BIN	Biological Institute, Botanical Institute
FTI	Institute of Physiotherapy
GONTI	State United Sci-Tech Press
GOST	All Union State Standard
GRRRI	State Roentgenology, Radiology, and Cancer Institute
GTTI	State Technical and Theoretical Literature Press
GU	State University
I Kh N	Scientific Research Institute of Surgical Neuropathology
IL (IIL)	Foreign Literature Press
IONKh	Inst. Gen. and Inorganic Chemistry (N. S. Kurnakov)
IP	Soil Science Inst. (Acad. Sci. USSR)
ISN (Izd. Sov. Nauk)	Soviet Science Press
Izd.	Press
LEM	Laboratory for experimental morphogenesis
LENDVI	Leningrad Inst. of Dermatology and Venereology
LEO	Laboratory of Experimental Zoology
LIKhT	Leningrad Surgical Institute for Tuberculosis and Bone and Joint Diseases
LIPZ	Leningrad Inst. for Study of Occupational Diseases
LIPK	Leningrad Blood Transfusion Institute
Medgiz	State Medical Literature Press
MOPISh	Moscow Society of Apiculture and Sericulture
MVI	Moscow Veterinary Institute
MZdrav	Ministry of Health
MZI	Moscow Zootechnical Institute
LOKhO	Leningrad Society of Orthopedic Surgeons
NIIZ	Scientific Research Institute of Zoology
NINKhI	Scientific Research Institute of Neurosurgery
NIU	Scientific Institute for Fertilizers
NIUIF	Scientific Research Institute of Fertilizers and Insecticides
NIVI	Veterinary Scientific Research Institute
ONTI	United Sci.-Tech. Press
OTI	Division of Technical Information
RBO	Russian Botanical Society
ROP	Russian Society of Pathologists
SANIIRI	Central Asia Scientific Research Institute of Irrigation
SANIISh	Central Asia Scientific Research Institute of Sericulture
TsNII	All-Union Central Scientific Research Institute
TsNTL	Central Scientific and Technical Laboratory
VASKhNIL	All-Union Academy of Agricultural Sciences
VIG	All-Union Institute of Helminthology
VIEM	All-Union Institute of Experimental Medicine
VIR	All-Union Institute of Plant Cultivation
VIUAA	All-Union Institute of Fertilizers, Soil Science, and Agricultural Engineering
VIZR	All-Union Institute of Medical and Pharmaceutical Herbs
VNIRO	All-Union Scientific Institute of Fishing and Oceanography
ZIN	Zoological Inst. (Acad. Sci. USSR)

Note: Abbreviations not on this list and not explained in the translation have been transliterated, no further information about their significance being available to us. - Publisher.

RUSSIAN JOURNALS FREQUENTLY CITED
[Biological Sciences]

Abbreviation*	Journal*	Translation
Agrobiol.	Agrobiologiia	Agrobiology
Akusherstvo i Ginekol.	Akusherstvo i Ginekologiia	Obstetrics and Gynecology
Antibiotiki	Antibiotiki	Antibiotics
Aptechnoe Delo	Aptechnoe Delo	Pharmaceutical Transactions
Arkh. Anat. Gistol. i Embriol.	Arkhiv Anatomii Gistologii i Embriologii	Archives of Anatomy, Histology, and Embryology
Arkh. Biol. Nauk SSSR	Arkhiv Biologicheskikh Nauk SSSR	Archives of Biological Science USSR
Arkh. Patol.	Arkhiv Patologii	Archives of Pathology
Biofizika	Biofizika	Biophysics
Biokhimiia	Biokhimiia	Biochemistry
Biokhim. Plodov i Ovoshchei	Biokhimiia Plodov i Ovoshchei	Biochemistry of Fruits and Vegetables
Bot. Zhur.	Botanicheskii Zhurnal	Journal of Botany
Biull. Eksptl. Biol. i Med.	Biulleten Eksperimentalnoi Biologii i Meditsiny	Bulletin of Experimental Biology and Medicine
Biull. Moskov. Obshchestva Ispytatelei Prirody, Otdel Biol.	Biulleten Moskovskogo Obshchestva Ispytatelei Prirody, Otdel Biologicheskii	Bulletin of the Moscow Naturalists Society, Division of Biology
Doklady Akad. Nauk SSSR	Doklady Akademii Nauk SSSR	Proceedings of the Academy of Sciences USSR
Eksptl. Khirurg.	Eksperimentalnaia Khirurgiia	Experimental Surgery
Farmakol. i Toksikol.	Farmakologiia i Toksikologiia	Pharmacology and Toxicology
Farmatsiia	Farmatsiia	Pharmacy
Fiziol. Rastenii	Fiziologiia Rastenii	Plant Physiology
Fiziol. Zhur. SSSR	Fiziologicheskii Zhurnal SSSR im. I. M. Sechenova	I. M. Sechenova Physiology Journal USSR
Gigiena i Sanit.	Gigiena i Sanitariia	Hygiene and Sanitation
Izvest. Akad. Nauk SSSR, Ser. Biol.	Izvestiia Akademii Nauk SSSR, Seriia Biologicheskaia	Bulletin of the Academy of Sciences USSR, Biology Series
Izvest. Tikhookeanskogo N. I. Inst. Rybnogo Khoz. i Okeanog.	Investiia Tikhookeanskogo N. I. Instituta Rybnogo Khoziaistva i Okeanografii	Bulletin of the Pacific Ocean Scientific Institute of Fisheries and Oceanography
Khirurgiia	Khirurgiia	Surgery
Klin. Med.	Klinicheskaia Meditsina	Clinical Medicine
Lab. Delo	Laboratornoe Delo(po Voprosam Meditsiny)	Laboratory Work (on Medical Problems)
Med. Parazitol.	Meditsinskaia Parazitologiia i Parazitarnye Bolezni	Medical Parasitology and Parasitic Diseases
Med. Radiol.	Meditsinskaia Radiologiia	Medical Radiology
Med. Zhur. Ukrain.	Medichnii Zhurnal Ukrainskii	Ukrainian Medical Journal
Mikrobiologiia	Mikrobiologiia	Microbiology
Mikrobiol. Zhur.	Mikrobiologichnii Zhurnal	Microbiology Journal
Nevropatol., Psikhiat. i Psikhogig.	Nevropatologiia, Psikhiatriia i Psikhogigiena	Neuropathology, Psychiatry and Psychohygiene
Ortoped., Travmatol. i Protez.	Ortopediia, Travmatologiia i Protezirovanie	Orthopedics, Traumatology and Prosthetics
Parazitol. Sbornik	Parazitologicheskii Sbornik	Parasitology Collection
Pediatriia	Pediatriia	Pediatrics
Pochvovedenie	Pochvovedenie	Soil Science
Priroda	Priroda	Nature
Problemy Endokrinol. i Gormonoterap.	Problemy Endokrinologii i Gormonoterapii	Problems of Endocrinology and Hormone Therapy
Problemy Gematol.	Problemy Gematologii i Perelivaniia Krovi	Problems of Hematology and Blood Transfusion
Problemy Tuberk.	Problemy Tuberkuleza	Problems of Tuberculosis
Sovet. Med.	Sovetskaia Meditsina	Soviet Medicine
Sovet. Vrachebny Zhur.	Sovetskii Vrachebnyi Zhurnal	Soviet Physicians Journals
Stomatologiia	Stomatologiia	Stomatology
Terap. Arkh.	Terapevticheskii Arkhiv	Therapeutic Archives
Trudy Gelmint. Lab.	Trudy Gelmintologicheskoi Laboratorii	Transactions of the Helminthology Laboratory
Trudy Inst. Genet.	Trudy Instituta Genetiki	Transactions of the Institute of Genetics

* LIBRARY OF CONGRESS TRANSLITERATION SYSTEM.

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Abbreviation	Journal	Translation
Trudy Inst. Gidrobiol.	Trudy Instituta Gidrobiologii	Transactions of the Institute of Hydrobiology
Trudy Inst. Mikrobiol.	Trudy Instituta Mikrobiologii	Transactions of the Institute of Microbiology
Trudy Inst. Okean.	Trudy Instituta Okeanologii, Akademii Nauk SSSR	Transactions of the Institute of Oceanology, Academy of Sciences, USSR
Trudy Leningrad Obshchestva Estestvoisp.	Trudy Leningrad Obshchestva Estestvoispytatelei	Transactions of the Leningrad Society of Naturalists
Trudy Vsesoiuz. Gidrobiol. Obshchestva	Trudy Vsesoiuznogo Gidrobiologicheskogo Obshchestva	Transactions of the All-Union Hydrobiological Society
Trudy Vsesoiuz. Inst. Eksptl. Med.	Trudy Vsesoiuznogo Instituta Eksperimentalnoi Meditsiny	Transactions of the All-Union Institute of Experimental Medicine
Ukrain. Biokhim. Zhur.	Ukrainskii Biokhimicheii Zhurnal	Ukrainian Biochemical Journal
Urologiia	Urologiia	Urology
Uspekhi Biokhimiia	Uspekhi Biokhimiia	Progress in Biochemistry
Uspekhi Sovremennoi Biol.	Uspekhi Sovremennoi Biologiia	Progress in Contemporary Biology
Vestnik Akad. Med. Nauk SSSR	Vestnik Akademii Meditsinskikh Nauk SSSR	Bulletin of the Academy of Medical Science USSR
Vestnik Khirurg. im. Grekova	Vestnik Khirurgii imeni Grekova	Grekov Bulletin of Surgery
Vestnik Leningrad. Univ. Ser. Biol.	Vestnik Leningradskogo Universiteta, Seriia Biologii	Journal of the Leningrad Univ., Biology Series
Vestnik Moskov. Univ., Ser. Biol. i Pochvov.	Vestnik Moskovskogo Universiteta, Seriia Biologii i Pochvovedeniia	Bulletin of the Moscow University, Biology and Soil Science Series
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